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# THE SURVIVAL AND DEVELOPMENT OF THE SHRIMP *CRANGON CRANGON* (L.), REARED IN THE LABORATORY UNDER NON-CIRCADIAN LIGHT–DARK CYCLES

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**Abstract:** The shrimp, *Crangon crangon* (L.), has been reared from hatching to the late juvenile phase, under a circadian (LD 12 : 12) and two non-circadian (LD 8 : 8 and LD random) light–dark cycles. During the zoeal phase, survival in the non-circadian regimes was markedly lower than in the control circadian treatments. This effect supports the hypothesis that the entraining influence of daily environmental cycles is important in coordinating the various physiological processes within the animal. There was no evidence, however, that growth and morphological development were affected in the non-circadian regimes.

## INTRODUCTION

Variations in the intensity, spectral composition and polarization of light have all been found to affect growth and reproduction in Crustacea (Buikema, 1968, 1973). It is the daily cycle of light and dark, and the seasonal changes in the proportions of light and dark in this cycle, however, which are most likely to be of significance in the life of crustaceans.

There are many reports that seasonal effects on growth, moulting, metabolic rate, induction of diapause, maturation of gonads and sex determination may be under photoperiodic control in some species (Passano, 1960; Aiken, 1969; Segal, 1970; Segerstråle, 1970; Rice & Armitage, 1974; Starkweather, 1976; Bülnheim, 1978). The effects of light cycles on crustacean growth, other than those related to seasonal photoperiodism have been less well studied. Some work has compared the effects of constant conditions with those of daily light–dark cycles, but the results often conflict. Some species have shown few differences in survival or moulting rates, when kept in constant conditions rather than in a daily light cycle (Crisp & Patel, 1960; Costlow & Bookhout, 1962; Bückman & Adelung, 1964; Buikema, 1973). In other cases, constant conditions have been found to be detrimental (Sandoz & Rodgers, 1944; Drach, 1947; Stephens, 1955; Bliss & Boyer, 1964; Auvray & Dussart, 1967; Reeve, 1969; Spindler, 1969; Starkweather, 1976), whilst in a single instance, survival was found to be greater in constant conditions than in a natural

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light cycle (Templeman, 1936). Present knowledge of the effects of light cycles on crustacean growth is therefore confused.

Much evidence now suggests that endogenous circadian rhythms, entrained by daily environmental cycles are ubiquitous in the physiological organization of eukaryotes. An animal's physiology may, therefore, be considered to comprise many rhythmic functions, where the entraining influence of daily environmental cycles is important in maintaining appropriate timing relationships between these functions and the environment, and also between the various functions themselves (Halberg, 1960a, b; Pittendrigh, 1960, 1961). Light cycles may therefore act on crustaceans principally through their entraining influence on endogenous, circadian rhythms. Theory suggests that animals reared in the absence of environmental cycles, or in cycles with periods greatly different from 24 h (non-circadian), will suffer detrimental effects because the proper coordination of their physiological processes would be impossible. Alternatively, individual endogenous, circadian rhythms can, within limits, be entrained to run with periods deviating from 24 h (Enright, 1965). It is possible, therefore, that exposure to environmental cycles with periods less than, but not greatly different from 24 h, might be beneficial because the animal's relative time-scale would be compressed and some life-history processes might be accelerated. Pittendrigh & Minis (1972), Saunders (1972) and Von Saint Paul & Aschoff (1978), have shown that non-circadian light–dark cycles are detrimental to the growth and survival of some insects. Dalley (1979, 1980), also found that the prawn *Palaemon elegans* suffered increased mortality, significant reductions in growth and changes in morphological development when reared under non-circadian light–dark cycles.

Further attempts to examine the relationship between light–dark cycles and crustacean growth are described here, using the brown shrimp *Crangon crangon* (L.) reared under non-circadian light–dark cycles.

## Materials and Methods

Ovigerous females of *C. crangon* were collected by beam-trawling in Port Erin Bay, Isle of Man, during January and February. Females were held individually at 20 °C in an LD 12 : 12 light regime in buckets containing 2 l of strongly aerated, filtered sea water and a substratum of 3 cm of clean sand. Pieces of the adductor muscle of the queen scallop (*Chlamys opercularis*) were given as food every 3 days and the water was changed every 6 days until the larvae hatched.

Larvae obtained in this way were used in three separate experiments, each experiment using only the larvae from a single brood. All experiments were conducted in light-tight cabinets constructed in a constant temperature room at 20 ± 0.5 °C. Each cabinet was lit by a Phillips 5 ft, 65–80 W, white fluorescent tube, giving ≈640 lux at the cabinet floor and controlled by a time-switch to produce square-wave light–dark cycles.

First, animals were reared from hatching to the first post-larval stage under an LD 12 : 12 and under an LD 8 : 8 light–dark cycle. Animals were kept individually in lidded, compartmented clear-polystyrene trays. Each tray had 24 compartments, each holding 100 ml of sea water (34‰ *S*, filtered through a Whatman GFC filter) and provided with gentle aeration. There were two trays in each treatment. Freshly hatched *Artemia* nauplii (San Francisco stock) were given as food at a concentration of not less than 10/ml, and during the last zoeal and first post-larval stage, these were supplemented with small pieces of the mantle margin of a freshly killed *Mytilus edulis*. Every third day animals were transferred to clean trays containing fresh sea water and food. Daily checks were made for dead specimens and moults. The morphological stages of newly-moulted animals were determined microscopically, and the total length of each newly-moulted animal measured, from the tip of the rostrum to the distal margin of the telson. Measurements were made to 0.06 mm on animals held still on a cavity-slide by the surface tension of a small drop of water, using a graduated eye-piece graticule fitted to a dissecting microscope.

Secondly, animals were reared from hatching to the late juvenile phase under LD 12 : 12, LD 8 : 8 and LD random light–dark cycles. The LD random cycle was devised by extracting two separate series of random numbers, varying between 6 and 18, from a random number table. Each member of the first series was taken to represent successive light portions of the light–dark cycle in hours, and each member of the second series represented the corresponding dark portions. A light–dark regime whose period was constantly varying between a possible maximum of 36 h (18 h light and 18 h dark) and a possible minimum of 12 h (6 h light and 6 h dark) was therefore produced, but with a mean period of $\approx$24 h and with the mean length of each light and dark portion of the cycle being $\approx$12 h. Animals therefore received approximately the same total amounts of light and dark in the LD random treatment as in the LD 12 : 12 treatment. During zoeal developments, animals were kept individually in compartmented trays, using the method in the first experiment. After metamorphosing, however, animals were kept in mass culture in lidded, polythene buckets holding 2 l of continuously aerated, filtered sea water, with a substratum of 3 cm of clean sand. Initially animals were kept at a density of $\approx$1 per 130–200 ml of sea water, but this was gradually increased to 1/l by the late juvenile phase. Every third day, pieces of the mantle margins of freshly killed *Mytilus* were provided in excess. Water was changed every 6th day and the sand every 12th day. During the zoeal phase deaths were recorded daily, but the total length of each animal was measured only immediately after metamorphosis and thereafter at 6- to 15-day intervals to the end of the experiment. Post-larvae and small juveniles were measured as in the first experiment. Larger juveniles were held flat on a glass-slide and measured with micrometer calipers to 0.1 mm.

In the third experiment, animals were also reared under LD 12 : 12, LD 8 : 8 and LD random light–dark cycles from hatching to the late juvenile phase, but in mass culture throughout. Zoeae were kept in lidded, polythene buckets containing 2 l of

gently aerated, filtered sea water. Freshly hatched *Artemia* nauplii were given as food at a concentration of 10/ml and every 3rd day animals were transferred to clean buckets with fresh sea water and *Artemia*. Initially there were 80 zoeae per bucket, with three buckets in each treatment. After metamorphosis animals were reared as in the second experiment, either in 2-l plastic buckets or 10-l clear polystyrene tanks. The total length of each animal was measured immediately after metamorphosis and thereafter at 6- to 15-day intervals to the end of the experiment.

After 100 days, the surviving animals in the second and third experiments were sexed, using the size and shape of the endopodites of the first pleopods and the presence of an appendix masculina on the endopodite of the second pleopod of the males, to distinguish the sexes (Lloyd & Yonge, 1947; Meredith, 1952; Tiews, 1967).

The division of the early development of *Crangon crangon* into zoeal, post-larval and juvenile phases, follows Gurney (1942).

## Results

### Experiment 1. Survival and growth of zoeal stages in LD 12 : 12 and LD 8 : 8

#### *Survival*

Survival during the zoeal phase was markedly lower in the LD 8 : 8 than in the LD 12 : 12 treatment. Significant differences ($P < 5\%$) were found between the percentage survival in each treatment, at each larval stage from zoea 4 onwards (Table I).

#### *Growth and moulting*

There were no differences between the mean sizes of each zoeal stage or in the mean times taken to reach each zoeal stage in the LD 12 : 12 and LD 8 : 8 regimes (Table I).

### Experiments 2 and 3. Long-term growth in LD 12 : 12, LD 8 : 8 and LD Random

#### *Survival*

In Experiment 2 survival to metamorphosis in LD 8 : 8 was lower than in LD 12 : 12 but significant differences ($P < 5\%$) were found only between the percentage survival in each treatment at Days 9 and 12 (Table II). Survival in LD Random was lower than in LD 8 : 8, and significantly lower than in LD 12 : 12 from Day 9 onwards ($P < 5\%$) (Table II, Fig. 1). There was a close agreement between the survival rates found in the LD 12 : 12 and LD 8 : 8 treatments in the first and second experiments (Fig. 1).

After metamorphosis in Experiment 2 and throughout Experiment 3, because

TABLE I

Experiment 1, *Crangon crangon*: A, percentage survival to each larval stage in each light regime, $d$ = ratio of sample difference between treatments to the SE of that difference, for $P = 0.05$, $d = 1.96$; B, total length of each larval stage in each treatment; C, time taken to reach each larval stage in each treatment.

| | | LD | Zoea 1 | Z2 | Z3 | Z4 | Z5 | Post-larva 1 |
|---|---|---|---|---|---|---|---|---|
| A | Survival (%) | 12 : 12 (Initial $n = 48$) | 100 | 89.6 | 85.4 | 81.3 | 77.1 | 70.8 |
| | | 8 : 8 (Initial $n = 48$) | 100 | 85.4 | 70.8 | 58.3 | 56.3 | 47.7 |
| | $d$ | | | 0.618 | 1.74 | 2.44 | 2.17 | 2.24 |
| B | Total length mm (mean ± SE) | 12 : 12 | 2.54 ± 0.004 | 2.89 ± 0.01 | 3.26 ± 0.02 | 3.73 ± 0.03 | 3.94 ± 0.06 | 3.99 ± 0.04 |
| | | 8 : 8 | 2.54 ± 0.006 | 2.91 ± 0.02 | 3.23 ± 0.02 | 3.68 ± 0.04 | 3.92 ± 0.05 | 4.02 ± 0.07 |
| C | Days to stage (mean ± SE) | 12 : 12 | 0 | 3.02 ± 0.02 | 6.12 ± 0.10 | 9.29 ± 0.09 | 11.94 ± 0.15 | 14.11 ± 0.25 |
| | | 8 : 8 | 0 | 3.00 ± 0 | 6.0 ± 0.80 | 9.55 ± 0.12 | 11.88 ± 0.22 | 14.20 ± 0.33 |

animals were not kept individually, mortalities could mostly be attributed to cannibalism, and it was not possible to determine what proportions, if any, were due to the light regimes.

TABLE II

Experiment 2, *Crangon crangon*: percentage survival to the approximate time of metamorphosis in each light regime; $d$ = ratio of sample difference between treatments to the SE of that difference, for $P = 0.05$, $d = 1.96$.

| | | Days | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | LD | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 |
| Survival (%) | 12 : 12 | 97.9 | 89.6 | 87.5 | 83.3 | 77.1 | 70.8 | 58.3 | 52.1 |
| | 8 : 8 | 95.8 | 81.3 | 70.8 | 64.6 | 60.4 | 52.1 | 47.9 | 45.8 |
| | ($d$, 12 : 12 v 8 : 8 = ) | (0.583) | (1.153) | (2.012) | (2.08) | (1.76) | (1.87) | (1.02) | (0.617) |
| | Random | 91.7 | 75.0 | 45.8 | 35.4 | 33.3 | 27.1 | 25.0 | 20.8 |
| | ($d$, 12 : 12 v Rand. = ) | (1.35) | (1.87) | (4.3) | (4.74) | (4.29) | (4.28) | (3.30) | (3.44) |

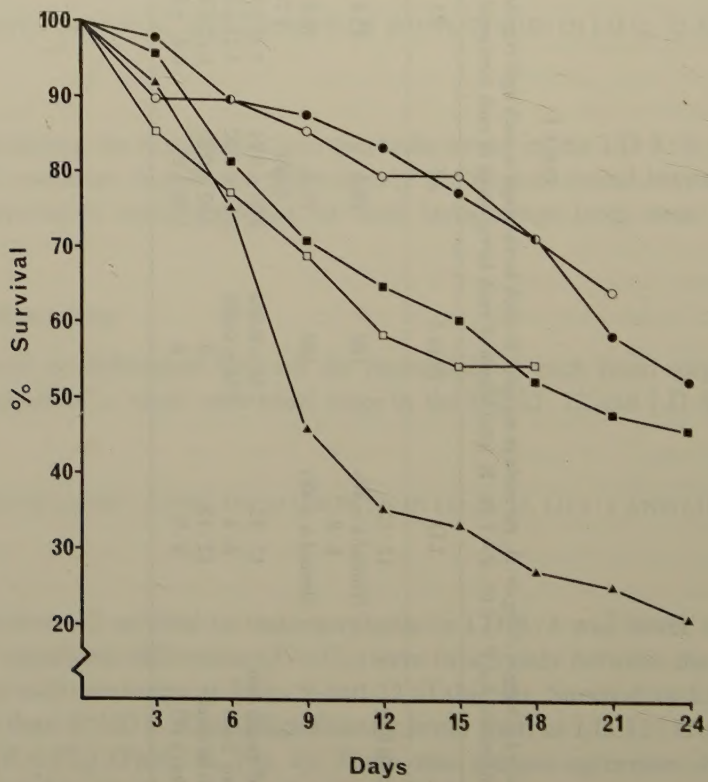


Fig. 1. The percentage survival of animals to the approximate time of metamorphosis in Experiment 2: ●, LD 12 : 12 (initial $n = 48$); ■, LD 8 : 8 (initial $n = 48$); ▲, LD random (initial $n = 48$); corresponding survival in LD 12 : 12 (○) and LD 8 : 8 (□) in Experiment 2.

*Growth*

Analysis of variance showed no significant differences ($P > 10\%$), between the mean total length of animals immediately after metamorphosis, or between the mean times taken to metamorphose in each treatment in the second and third experiments (Table III).

TABLE III

Experiments 2 and 3, *Crangon crangon*: total length at metamorphosis and time taken to metamorphose in each light regime.

| | | LD 12 : 12 | LD 8 : 8 | LD random |
|---|---|---|---|---|
| Total length mm. | Expt. 2 | 3.88 ± 0.04 | 3.84 ± 0.06 | 3.91 ± 0.09 |
| (Mean ± SE) | Expt. 3 | 3.95 ± 0.02 | 3.85 ± 0.02 | 3.89 ± 0.02 |
| Days to metamorphosis | Expt. 2 | 20.48 ± 0.39 | 20.85 ± 0.66 | 21.22 ± 1.04 |
| (Mean ± SE) | Expt. 3 | 23.14 ± 0.41 | 23.78 ± 0.33 | 24.52 ± 0.34 |

The subsequent growth of animals after metamorphosis (measured as total length), also showed few differences between treatments in the second and third experiments (Table IV), although growth was generally the lowest in the LD Random regime.

*Sex ratios*

Rochanaburanon (1974) claimed that animals in laboratory culture first showed sexual dimorphism at the seventh post-larval stage, at a total length of 10.5–12.6 mm. Muus (1967) found that in natural populations in Niva Bay, Denmark, the appendix masculina was clearly visible on specimens of 15–16 mm total length, but Lloyd & Yonge (1947), Meredith (1952) and Tiews (1967) found that the appendix masculina only became apparent in animals >20 mm total length and even then was very difficult to distinguish from the small swelling between the base of the endopodite and its rather flattened end, in animals <30 mm. The differences in the size and shape of the endopodite of the first pleopod (Lloyd & Yonge, 1947), were more useful in distinguishing the sex of smaller specimens, but this was always difficult in animals <22 mm total length. Table V shows that the total number of animals surviving to a size at which they could be sexed, after ≈100 days, in Experiments 2 and 3 was extremely low. Very high mortalities occurred after animals had reached 15–18 mm total length, due mainly to the extreme cannibalistic tendencies of the animals, which became greater as the animals grew larger. From the data available, the sex ratio in the LD 12 : 12 treatment favoured females, that in LD 8 : 8 was approximately 1 : 1, and the ratio in the LD Random treatment appeared to be shifted towards maleness. The numbers of each sex in each treatment were compared using Fisher's

TABLE IV

Experiments 2 and 3, *Crangon crangon*: total length from metamorphosis to the late juvenile phase in each treatment.

| | | | Days | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | LD | 36 | 43 | 50 | 56 | 63 | 74 | 87 |
| Total length (mm) (Mean ± SE) | Expt. 2 | 12 : 12 | 7.42 ± 0.04 | 9.23 ± 0.36 | 10.95 ± 0.48 | 13.31 ± 0.51 | 14.91 ± 0.76 | 19.39 ± 1.06 | 23.39 ± 1.44 |
| | | 8 : 8 | 7.75 ± 0.41 | 9.34 ± 0.47 | 11.92 ± 0.60 | 13.59 ± 0.73 | 15.54 ± 0.81 | 19.94 ± 1.27 | 25.61 ± 1.71 |
| | | Random | 8.13 ± 0.70 | 9.5 ± 0.94 | 11.4 ± 1.15 | 12.82 ± 0.14 | 14.02 ± 1.64 | 17.79 ± 1.16 | 20.5 ± 0.86 |
| | | | Days | | | | | | |
| | | LD | 36 | 52 | 66 | 76 | 86 | | |
| Total length (mm) (Mean ± SE) | Expt. 3 | 12 : 12 | 6.79 ± 0.21 | 11.13 ± 0.47 | 15.22 ± 0.62 | 19.15 ± 1.04 | 24.1 ± 1.48 | | |
| | | 8 : 8 | 6.43 ± 0.14 | 10.27 ± 0.30 | 14.76 ± 0.46 | 18.94 ± 0.75 | 24.19 ± 1.18 | | |
| | | Random | 6.04 ± 0.14 | 9.24 ± 0.33 | 12.73 ± 0.43 | 16.45 ± 0.68 | 22.18 ± 0.82 | | |

test for small, random, binomial samples (Langley, 1968), but no significant differences were found between the numbers in any treatment ($P > 5\%$).

TABLE V

Experiments 2 and 3, *Crangon crangon*: the sex of animals surviving after 100 days; figures in parentheses are percentages.

| LD | *n* | Male | Female |
|---|---|---|---|
| 12 : 12 | 13 | 5 (38.46) | 8 (61.54) |
| 8 : 8 | 11 | 6 (54.55) | 5 (45.45) |
| Random | 17 | 12 (70.59) | 5 (29.41) |

## DISCUSSION

The animals in these experiments were clearly affected by the periodicity of the environmental light to which they were exposed, in a way predicted by rhythm theory. As expected, survival in the non-circadian (LD 8 : 8 and LD Random) regimes, and particularly in the LD random regimes, was reduced compared to the control circadian treatments (LD 12 : 12) (Tables I, II, Fig. 1). This evidence strongly suggests that as in *Palaemon elegans* (Dalley, 1979, 1980), the physiology of *Crangon crangon* is organized with a circadian periodicity. The overall efficiency of the animal's physiology is primarily dependent on the correct synchronization, by circadian environmental cycles, of the endogenous, circadian rhythms controlling its various physiological processes. The periodicities of both the LD 8 : 8 and LD random regimes were too far removed from 24 h for synchronization to take place, and the ensuing high mortalities may have resulted from the predicted loss of physiological coordination. As with *Palaemon* (Dalley, 1979), the mortality effects produced by the two non-circadian cycles were not identical, the effect in LD Random being much greater (Table II, Fig. 1). The reason for this difference might be that, although environmental cycles are important in synchronizing the diverse circadian rhythms in the animal's physiology, individual rhythmic functions are capable of synchronizing each other, perhaps by chemical means (Pittendrigh, 1960; Bünning, 1967). In the absence of circadian environmental cycles, or in the regular LD 8 : 8 regime, animals may be capable of maintaining functional organizations using such processes of mutual phasing. In the completely disrupted LD Random regime, however, the constantly changing period of the external light cycle may override any endogenous phasing mechanisms to a greater extent.

In contrast to *Palaemon* the experiments did not provide significant evidence of any other effects resulting from exposure to the non-circadian light regimes, apart from the high mortality during the zoeal stages. There were no differences in growth or moulting rates throughout the experiments (Tables I, III and IV). Also, the

low numbers of animals surviving to be sexed in the second and third experiments did not provide sufficient evidence to show that the non-circadian light regimes significantly altered the sex ratios (Table V). There was an indication, however, that as in *Palaemon* (Dalley, 1979), exposure to non-circadian regimes favoured maleness, the effect being greatest in the LD random regime.

In agreement with Meixner (1966a, b) and Rochanaburanon (1974), there was no evidence that the number of zoeal stages is labile in *Crangon*.

Results of other workers where constant conditions have been found to induce detrimental effects in crustaceans (Sandoz & Rodgers, 1944; Drach, 1947; Stephens, 1955; Bliss & Boyer, 1964; Auvray & Dussart, 1967; Reeve, 1969; Spindler, 1969; Starkweather, 1976), could also be interpreted using rhythm theory, because animals in constant conditions are exposed to similar stresses as those under non-circadian regimes. That is, they must achieve a functional organisation of the diverse rhythmic processes comprising their physiology, in the absence of the synchronizing influence of daily environmental cycles. Furthermore, an increasing amount of evidence suggests that endogenous, circadian rhythms are the underlying mechanisms in many photoperiodic responses (Bünning, 1959; Pittendrigh & Minis, 1964; Pittendrigh, 1972). Animals have evolved a complex circadian organization of their physiological processes, as an adaptation to the daily cycles in the environment. It would be surprising, therefore, if this circadian organization were not involved in both short- and long-term effects of cyclic light on the gross process of growth and development, as evidence now suggests is the case.

The possibility that growth processes could be accelerated by rearing animals under light–dark cycles with periods less than, but not far removed from, 24 h, remains to be systematically investigated. Von Saint Paul & Aschoff (1978), did not find that longevity of the blowfly *Phormia terraenovae* was altered when reared under such non-circadian light–dark cycles. Dalley (1979), however, found some evidence that zoeae of *Palaemon elegans* metamorphosed after fewer than the expected number of moults in an LD 8 : 8 regime.

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## References

Aiken, D. E., 1969. Photoperiod, endocrinology and the crustacean molt cycle. *Science, N.Y.*, Vol. 164, pp. 149–155.

Auvray, C. & B. Dussart, 1967. Rôle de quelques facteurs du milieu sur le développement post-embryonnaire des cyclopides (Crustacés, Copépodes). II. Cas des *Cyclops* et influences des facteurs extérieurs. *Bull. Soc. zool. Fr.*, Vol. 92, pp. 11–22.

Bliss, D.E. & J.R. Boyfr, 1964. Environmental regulation of growth in the decapod crustacean *Gecarcinus lateralis*. *Gen. compar. Endocr.*, Vol. 4, pp. 15–41.

Bückman, D. & D. Adelung, 1964. Der Einfluss der Umweltfaktoren auf das Wachstum und den Häutungsrhythmus der Strandkrabbe *Carcinides maenas*. *Helgoländer wiss. Meeresunters.*, Vol. 10, pp. 91–103.

Buikema, A.L., 1968. Effects of varying wavelengths, intensities and polarized light on population dynamics and ephippial production of *Daphnia pulex* Leydig, 1860, emend. Richard (1896), (Cladocera). *Crustaceana*, Vol. 14, pp. 45–55.

Buikema, A.L., 1973. Some effects of light on the growth, moulting, reproduction and survival of the cladoceran, *Daphnia pulex*. *Hydrobiologia*, Vol. 41, pp. 391–418.

Bülnheim, H.P., 1978. Interaction between genetic, external and parasitic factors in sex determination of the crustacean amphipod, *Gammarus duebeni*. *Helgoländer wiss. Meeresunters.*, Vol. 31, pp. 1 33.

Bünning, E., 1959. Physiological mechanism and biological importance of the endogenous diurnal periodicity in plants and animals. In, *Photoperiodism and related phenomena in plants and animals*, edited by R.B. Withrow, Publication No. 55 of American Association for the Advancement of Science, Washington D.C., pp. 507–530.

Bünning, E., 1967. *The physiological clock*. Longmans/Springer-Verlag, New York, 167 pp.

Costlow, J.D. & C.G. Bookhout, 1962. The larval development of *Sesarma reticulatum* Say reared in the laboratory. *Crustaceana*, Vol. 4, pp. 281–294.

Crisp, D.J. & B.S. Patel, 1960. The moulting cycle of *Balanus balanoides* (L.). *Biol. Bull. mar. biol. Lab., Woods Hole*, Vol. 118, pp. 31–47.

Dalley, R., 1979. Effects of non-circadian light cycles on the survival and development of *Palaemon elegans* Rathke reared in the laboratory. In, *Cyclic phenomena in marine plants and animals*, *Proc. 13th Europ. mar. Biol. Symp.*, edited by E. Naylor & R.G. Hartnoll, Pergamon Press, London, pp. 157–163.

Dalley, R., 1980. Effects of non-circadian light cycles on the growth and moulting of *Palaemon elegans* Rathke, reared in the laboratory. *Mar. Biol.*, in press.

Drach, P., 1947. Lumière, glande du sinus et vitesse de parcours des intermues chez *Leander serratus* (Pennant). *C.r. hebd. Seanc. Acad. Sci., Paris*, Vol. 225, pp. 1376–1378.

Enright, J.T., 1965. Synchronization and ranges of entrainment. In, *Circadian clocks*, edited by J. Aschoff, North-Holland, Amsterdam, pp. 112–124.

Gurney, R., 1942. *Larvae of decapod crustacea*. Ray Society, London, 306 pp.

Halberg, F., 1960a. Temporal coordination of physiologic function. *Cold Spring Harb. Symp. quant. Biol.*, Vol. 25, pp. 289–310.

Halberg, F., 1960b. The 24 hour scale: a time dimension of adaptive functional organization. *Perspect. Biol. Med.*, Vol. 3, pp. 491–527.

Langley, R., 1968. *Practical statistics*. Pan Books, London, 399 pp.

Lloyd, A.J. & C.M. Yonge, 1947. The biology of *Crangon vulgaris* L. in the Bristol Channel and Severn Estuary. *J. mar. biol. Ass. U.K.*, Vol. 26, pp. 626–661.

Meixner, R., 1966a. Eine Methode zur Aufzucht von *Crangon crangon* (L.) (Crust. Decap. Natantia). *Arch. FischWiss.*, Vol. 17, pp. 1–4.

Meixner, R., 1966b. The effects of food supply on moulting, growth and spawning of the shrimp *Crangon crangon* (L.). *I.C.E.S., C.M., M: 5, Shellfish committee*, pp. 1–7.

Meredith, S.S., 1952. A study of *Crangon vulgaris* in the Liverpool Bay area. *Proc. Trans. Lpool biol. Soc.*, Vol. 58, pp. 75–109.

Muus, B.J., 1967. The fauna of Danish estuaries and lagoons. *Meddr Kommn Danm. Fisk.- og Havunders., Ny Serie*, Vol. 5 (1), pp. 1–316.

Passano, L.M., 1960. Moulting and its control. In, *The physiology of the crustacea, Vol. 1*, edited by T.H. Waterman, Academic Press, London, pp. 473–527.

Pittendrigh, C.S., 1960. Circadian rhythms and the circadian organization of living systems. *Cold Spring Harb. Symp. quant. Biol.*, Vol. 25, pp. 159–184.

Pittendrigh, C.S., 1961. On temporal organization in living systems. *Harvey Lect.*, Vol. 56, pp. 93–125.

Pittendrigh, C.S., 1972. Circadian surfaces and the diversity of possible roles of circadian organization in photoperiodic induction. *Proc. natn. Acad. Sci. U.S.A.*, Vol. 69, pp. 2734–2737.

Pittendrigh, C.S. & D.H. Minis, 1964. The entrainment of circadian oscillations by light and their role as photoperiodic clocks. *Am. Nat.*, Vol. 98, pp. 261–294.

PITTENDRIGH, C.S. & D.H. MINIS, 1972. Circadian systems: longevity as a function of circadian resonance in *Drosophila melanogaster*. *Proc. natn. Acad. Sci. U.S.A.*, Vol. 69, pp. 1537–1539.

REEVE, M.R., 1969. The laboratory culture of the prawn *Palaemon serratus*. *Fishery Invest., Lond. Ser. 2*, Vol. 26 (11), pp. 1–36.

RICE, P.A. & K.B. ARMITAGE, 1974. The influence of photoperiod on processes associated with moulting in the crayfish *Orconectes nais* (Faxon). *Comp. Biochem. Physiol.*, Vol. 47A, pp. 243–259.

ROCHANABURANON, T., 1974. *Experimental rearing of some Irish Sea Caridea*. Ph.D. thesis, University of Liverpool, 111 pp.

SANDOZ, M. & R. RODGERS, 1944. The effect of environmental factors on hatching, moulting and survival of zoeae larvae of the blue crab, *Callinectes sapidus* Rathbun. *Ecology*, Vol. 25, pp. 216–228.

SAUNDERS, D.S., 1972. Circadian control of larval growth rate in *Sarcophaga argyrostoma*. *Proc. natn. Acad. Sci. U.S.A.*, Vol. 69, pp. 2738–2740.

SEGAL, E., 1970. Light, invertebrates. In, *Marine ecology*, *Vol. 1*, edited by O. Kinne, Wiley-Interscience, London, pp. 159–211.

SEGERSTRÅLE, S.G., 1970. Light control of the reproductive cycle of *Pontoporeia affinis* Lindström (Crustacea, Amphipoda). *J. exp. mar. Biol. Ecol.*, Vol. 5, pp. 272–275.

SPINDLER, K.D., 1969. Die Bedeutung der Photoperiode für die Entwicklung von *Cyclops vicinus*. *Zool. Anz. Suppl.*, Vol. 33, pp. 190–195.

STARKWEATHER, P.L., 1976. Influences of light regime on post-embryonic development in two strains of *Daphnia pulex*. *Limnol. Oceanogr.*, Vol. 21, pp. 830–837.

STEPHENS, G.C., 1955. Induction of moulting in the crayfish *Cambarus*, by modification of daily photoperiod. *Biol. Bull. mar. biol. Lab., Woods Hole*, Vol. 182, pp. 235–241.

TEMPLEMAN, W., 1936. The influence of temperature, salinity, light and food conditions on the survival and growth of the larvae of the lobster (*Homarus americanus*). *J. biol. Bd Can.*, Vol. 2, pp. 485–497.

TIEWS, K., 1967. Synopsis of biological data on the common shrimp *Crangon crangon* (Linnaeus, 1758). *F.A.O. Fish Rep.*, Vol. 57, pp. 1167–1224.

VON SAINT PAUL, U. & J. ASCHOFF, 1978. Longevity among blowflies *Phormia terraenovae* R.D. kept in non-24 hour light/dark cycles. *J. comp. Physiol.*, Vol. 127A, pp. 191–195.

*J. exp. mar. Biol. Ecol.*, 1980, Vol. 47, pp. 113–126
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# PHYSIOLOGICAL RESPONSE TO CHLORINATION OF THE UNICELLULAR MARINE ALGA *DUNALIELLA PRIMOLECTA* Butcher

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**Abstract:** The effect of static chlorination on the metabolism of *Dunaliella primolecta* Butcher has been investigated in terms of oxygen exchanges, uptake and evolution of phosphate ions. The immediate effects of chlorine on photosynthesis and respiration appear to be dependent on light: when algae were kept in the dark, chlorinations induced a decrease in respiration proportional to mortality, while chlorinations performed during a light dark cycle reduced only the photosynthetic rate for mortalities $\leqq 40\%$.

Small amounts of chlorine, although inducing no mortality, significantly reduced the phosphate uptake. Higher chlorine concentrations caused a leakage of $PO_4^{3-}$ ions but, after a time depending on chlorine concentration, the phosphate absorption rate in the surviving chlorinated cells and in their respective control cells was similar.

The measurement of acid-soluble fractions of organic and inorganic phosphorus suggested that metabolism was primarily affected by the action of chlorine. One hour after chlorination, which induced a 50% mortality, the intracellular ATP content was reduced by 70% and the formation of other phosphorylated compounds was completely inhibited. Twenty-four hours after this static chlorination, however, the surviving cells showed no apparent metabolic alteration.

## INTRODUCTION

Chlorine, added to the cooling system of seaside power plants to destroy fouling organisms, has a well-known environmental impact, in particular on the dragged phytoplankton. Indeed, many surveys have shown a serious decline in primary production at discharge points.

Chlorine reactions in sea water are now well known: chlorine added as $Cl_2$ or NaOCl first reacts with the bromide ions of the medium (0.8 mM) to form, at a low ammonia level, a mixture of HOBr and $BrO^-$ (Wong & Davidson, 1977). When the

chlorine concentration is not in excess with respect to ammonia concentration, free halogens react with ammonia to give monochloramine ($NH_2Cl$), monobromamine ($NH_2Br$), and dibromamine ($NHBr_2$), but monochloramine is more persistent than bromamines (Inmann & Johnson, 1978; Peron & Courtot, 1979). Chlorine also reacts with organic compounds of the medium (Jolley *et al.*, 1978).

To observe the influence of chlorination at cell level, toxicity assays have already been made by adding chlorine to cultures of *Dunaliella primolecta* (Videau *et al.*, 1979). With those static chlorinations, the filtered sea water used was poor in ammonium ( $< 0.002$ ppm $NH_4^+$ ) and, consequently, only the effect of free halogens was tested. The results showed, in particular, a higher sensitivity of the alga to chlorination performed in the light and the unavailing influence of halogens as long as the cells were kept in the dark.

As regards the specific action of bromine and chlorine on *Chlorella* (Kott & Edlis, 1969) one may think that bromine is the active halogen, as was suggested by the chemical analysis. The growth of the surviving chlorinated cells, however, appeared to be unaffected and one may consider that algal cells had kept their metabolic activity. Apart from photosynthetic measurements (Eppley *et al.*, 1976; Khalanski, 1977), only Toetz *et al.* (1977), to our knowledge, investigated the influence of chlorination on the physiological response of phytoplankton. They found that the absorption of inorganic nitrogen decreased by 50% after light chlorination (0.028 ppm) of lake water. But those results were obtained in a freshwater environment (chlorine, but no bromine action) and focused only on the immediate effect of chlorination.

The purpose of the present study was to assess both the immediate and long-term effects of lethal and sublethal chlorine concentrations on some metabolic aspects, such as oxygen exchanges, phosphate uptake and the development of phosphorylated compounds in *Dunaliella primolecta* Butcher, a marine phytoplanktonic alga (Chlorophyceae).

## Material and Methods

The algae were grown axenically in a Conway medium (Walne, 1966) under constant illumination (0.01 kcal $\cdot m^{-2} \cdot h^{-1}$) at $20 \pm 1$ °C.

### Chlorination procedures

In order to reduce the chlorine demand of the medium, algae were removed from the culture medium by centrifugation at 10000 *g* for 15 min, then put in sea water which had been filtered through a membrane filter LMR/7 (0.2 $\mu$m). Initial cell density is measured by means of a Coultronic model DI coulter-counter or a Malassez hemacytometer. The algal suspension was divided in two lots and chlorine was added directly as sodium hypochlorite in one batch culture; the other

one was used as the control. Added chlorine concentrations were 0.1 to 0.8 ppm for obtaining various mortalities in relation to initial cell density. The disappearance of free oxidant was followed by the colorimetric D.P.D. method. After a 6-h chlorination, residual oxidant was never detected in the cell culture under these conditions. Mortality was assessed by comparing survival cell number with initial one 24 h after chlorination (for more details about culture and chlorination procedures, see Videau *et al.*, 1979). The effect of halogen oxidant studied here was the result of a static chlorination in sea water without ammonium ($NH_4^+ <$ 0.002 ppm). Consequently, there was no formation of haloamine and only the action of bromine was tested.

### OXYGEN EXCHANGE MEASUREMENTS

To measure the oxygen, a Beckman model 778 $pO_2$ analyser was connected with a chart recorder and kept in contact with a cell culture stirred with a magnetic rod. The cells were maintained at constant temperature (20 °C ± 0.2) keeping the water flowing through a cooling jacket with a circulating pump. Chlorine was directly put into the test bottle with a syringe, through a rubber stopper. Photosynthesis measurements were made under an illumination of $2 \times 10^{-3}$ W · cm$^{-2}$. A blank was obtained by adding chlorine to filtered sea water. With the low chlorine percentage used, no deflection was observed on the recorded curves.

### PHOSPHATE ABSORPTION DETERMINATION

Phosphate absorption was followed by labelling the filtered sea water with a tracer, viz. the $^{32}P$ radioisotope (20 $\mu$Ci · l$^{-1}$) provided as $H_3{}^{32}PO_4$. The algae, removed from the culture medium, were washed twice by two 10-min centrifugations at 10 000 *g*. The pellet was adjusted to 2 ml with filtered sea water and poured into 248 ml of $^{32}P$-labelled sea water, chlorinated or not (control sample). Absorption was estimated from the filter activity; 10 ml of the labelled cell suspension were sampled and quickly filtered through a Whatman GF/C glassfiber filter (0.45 $\mu$m), rinsed with sea water at 0 °C. Algal activity was measured with a Geiger–Müller counter, the filter radioactivity containment being taken into account. As, however, the phosphate absorption rate and percentage are seriously reduced immediately after centrifugation (Fig. 1) chlorine was only added after a 2-h rest. Experiments were carried out at 17 °C ± 1 under $1.3 \times 10^{-3}$W · cm$^{-2}$ illumination and with bubbling aeration.

Although natural sea-water phosphate is a limiting factor in absorption kinetics, we chose to work in a natural environment, the absorption time being reduced to 30 min.

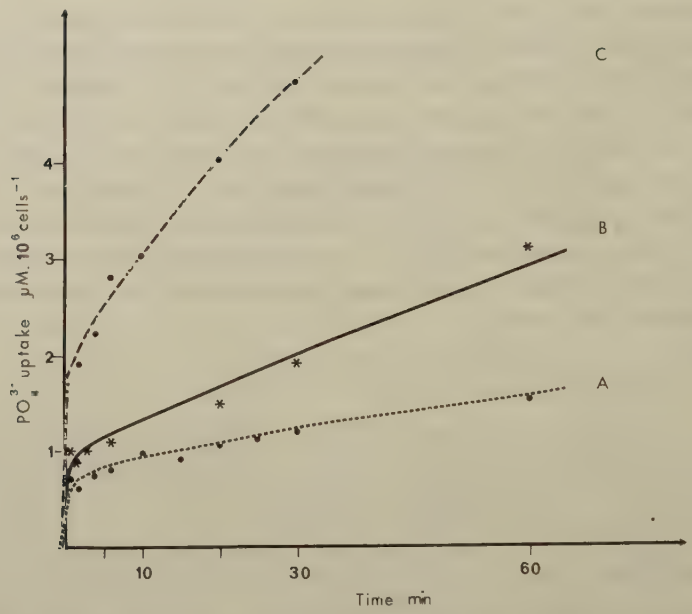


Fig. 1. Influence of recovery time after centrifugation on phosphate uptake by *Dunaliella primolecta*: A, 5 min; B, 30 min; C, 2 h.

## PHOSPHORYLATED COMPOUND ANALYSIS

The cells were put in a $^{32}P$-labelled medium, as previously described. Phosphorylated compounds were extracted from the algae filters by 10% TCA at 0 °C for 30 min. Inorganic phosphorus in the acid-soluble fraction was separated from organic phosphorus according to Ducet & Vandewalle's method (1959). The concentrations were deduced from radioactivity measurements. The separation of organic compounds was obtained by two-dimensional paper chromatography according to the procedure of Cole & Ross (1966). The initial extract was concentrated by vacuum evaporation at 30 °C, and adjusted to 200 µl with distilled water. Five to 10 µl of this extract were deposited on Whatman 3 MM chromatographic paper. Autoradiographies were obtained by exposure of the chromatograms for 3 to 15 days to a Kodak Regulix film. Phosphorylated compounds were identified either by reference to Cole & Ross (1966) and Floc'h & Penot (1978) chromatograms or by comparison with pure reference compound chromatographs. ATP cell concentrations were also determined by the firefly bioluminescent procedure described by Holm-Hansen Booth (1966), with a Lumac 1030 cell tester.

## RESULTS

The results given below are those of three series of experiments at least, or their mean. The "$Cl_2$" symbols used on the figures apply to chlorinated cells. Because the cell density and biological variations influence on the chlorine toxicity within cultures, the chlorination effects on metabolism will be expressed in regard to mortality.

### OXYGEN EXCHANGES

#### *Respiration*

In the first series of experiments, respiration alone was measured before and after chlorination of *Dunaliella* cells. In that case, the algae were kept in the dark during the whole test. When the mortality is below 20%, there is no chlorine influence on respiratory metabolism. Beyond 20% mortality, the decrease in respiration is proportional to the mortality (Table I).

TABLE I

Respiration in $\mu l\ O_2 \cdot 10^6\ cells^{-1} \cdot h^{-1}$ of *Dunaliella primolecta* before and immediately after chlorination: variation and mortality are given in percentages and cell density in $10^6\ cells \cdot ml^{-1}$.

| Initial cell density | Respiration | | Variation | Mortality |
|---|---|---|---|---|
| | Before $Cl_2$ | After $Cl_2$ | | |
| 0.8 | 0.69 | 0.93 | + 35 | 12 |
| 0.4 | 0.85 | 0.85 | 0 | 17 |
| 0.2 | 2.5 | 1.25 | − 50 | 40 |
| 0.8 | 0.67 | 0.32 | − 52 | 54 |
| 0.15 | 0.49 | 0.28 | − 43 | 58 |
| 0.15 | 0.52 | 0.2 | − 62 | 60 |
| 0.6 | 0.92 | 0 | − 100 | 66 |

#### *Photosynthesis and respiration*

As compared with the previous chlorination experiments made under different light conditions, the photosynthesis and respiration tests were performed either during the dark phase (Table II) or light phase (Table III), of the experiment.

Whatever the lighting phase, chlorinations induced only a decrease in photosynthetic rate when mortalities are $\leqq$ 40% although a significant decline in respiration was noted (Table II) when the algae were not subject to a light–dark cycle. For higher mortalities, the photosynthetic rate was always more reduced than the respiration rate.

TABLE II

Respiration (R) and photosynthesis (P) in $\mu l\ O_2 \cdot 10^6\ cells^{-1} \cdot h^{-1}$ and their variation immediately after chlorination in dark phase: variation and mortality are given in percentages and cell density in $10^6$ cells · $ml^{-1}$.

| Initial cell density | Before chlorination | | After chlorination | | Variation | | Mortality |
|---|---|---|---|---|---|---|---|
| | R | P | R | P | R | P | |
| 0.65 | 0.92 | 3.2 | 0.92 | 2.44 | 0 | 24 | 0 |
| 1.5 | 0.43 | 3.2 | 0.43 | 3.26 | 0 | 0 | 24 |
| 1.4 | 0.35 | 4.31 | 0.45 | 3.02 | +28 | −30 | 35 |
| 0.56 | 1.04 | 5.8 | 0.76 | 0.76 | −25 | −85 | 68 |
| 0.45 | 1.56 | 8.26 | 0.44 | 0.88 | −72 | −90 | 70 |
| 2.8 | 0.27 | 1.10 | 0.27 | 0.27 | 0 | −75 | 80 |
| 0.27 | 1.96 | 13.8 | 0 | 0 | −100 | −100 | 84 |

TABLE III

Respiration (R) and photosynthesis (P) in $\mu l\ O_2 \cdot 10^6\ cells^{-1} \cdot h^{-1}$ and their variation immediately after chlorination in light phase: variation and mortality are given in percentages and cell density in $10^6$ cells · $ml^{-1}$.

| Initial cell density | Before chlorination | | After chlorination | | Variation | | Mortality |
|---|---|---|---|---|---|---|---|
| | R | P | R | P | R | P | |
| 1.2 | 0.68 | 2.04 | 0.68 | 1.83 | 0 | −11 | 0 |
| 1 | 0.97 | 3.55 | 0.97 | 2.26 | 0 | −37 | 0 |
| 1.7 | 0.55 | 2.28 | 0.56 | 1.18 | 0 | −49 | 36 |
| 1.2 | 0.54 | 3.26 | 0.54 | 1.18 | 0 | −64 | 42 |
| 1.3 | 0.50 | 2.49 | 0.50 | 1.74 | 0 | −30 | 42 |
| 1.2 | 0.68 | 1.78 | 0.17 | 0.17 | −75 | −91 | 68 |

## PHOSPHATE ABSORPTION

### *Immediate result*

Whatever the mortality induced by chlorination, there was no significant difference, within the first 30 s, between the amount of phosphate ions absorbed by the various samples (Fig. 2). Ions were probably "fixed" in the apparent free space. With low mortality (30%) the absorption was first inhibited (Fig. 2A) followed by a slight leakage and then by a recovery of uptake at constant rate (Fig. 2B). A low chlorine concentration (0.1 ppm) inducing no subsequent mortality, decreases the uptake amount and rate, whereas with a high mortality, there was a serious leakage due to bursting cells.

*Long-term action*

To follow the long-term action of chlorine on phosphate absorption, the algae were divided in two batches with the same high cell density ($10^6$ cells · $ml^{-1}$). One batch was chlorinated (0.7 ppm). An aliquot volume of each culture was respectively added to several $^{32}P$-labelled sea-water samples, 1, 6, and 29 h after

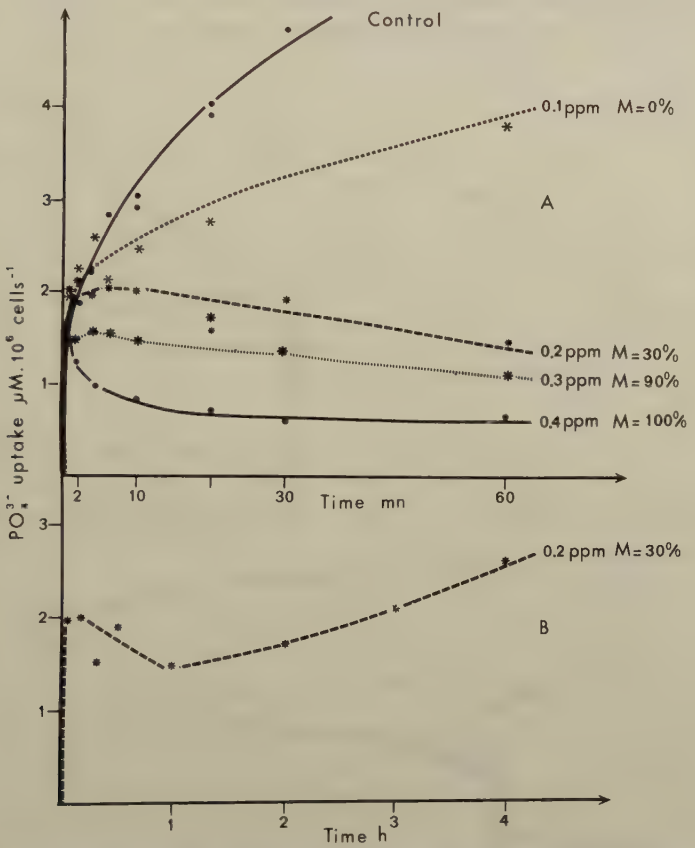


Fig. 2. Influence of chlorination on phosphate uptake: A, immediately after chlorination (<60 min); B, further development (mortality = 30%); M = mortality.

chlorination. With a mortality of 80%, phosphate uptake was, as before, inhibited during the first few hours (Fig. 3A, B) but 29 h after chlorine addition, absorption by the surviving cells was not significantly different from that of the control samples.

## PHOSPHORYLATED COMPOUND EVOLUTION

The evolution of phosphorylated compounds was observed in order to find out (a) the action of chlorination on membrane permeability or on metabolism, by

measurement of the acid-soluble fractions of inorganic (Pi) and organic (Po) phosphorus in the cells, and (b) the inhibiting influence of chlorination on the formation of phosphorylated intermediate products.

*Evolution of the inorganic and organic phosphorus fractions*

During the first minute, chlorination does not alter the Pi and Po amounts in chlorinated cells, when the mortality is close to 50% (Fig. 4A). The influence of

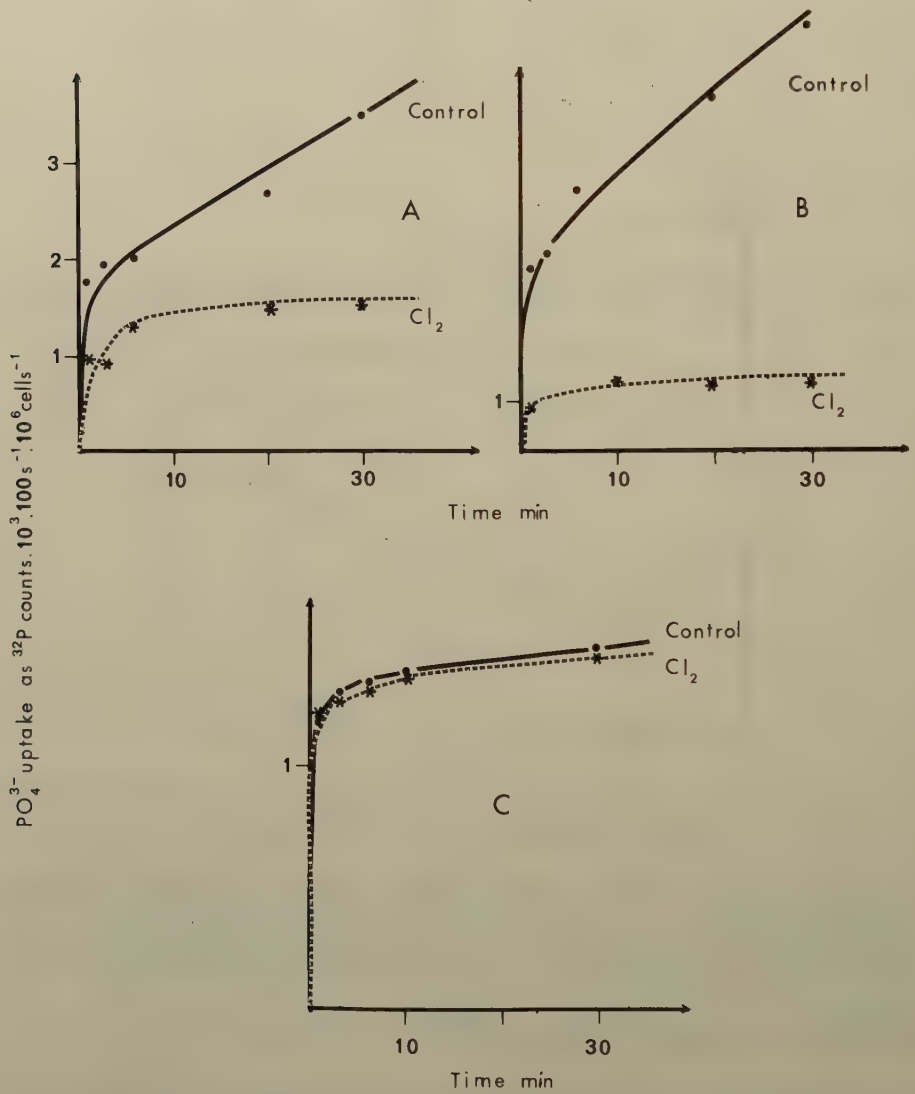


Fig. 3. Long-term effect of high chlorination (mortality = 80%) on phosphate uptake: A, 1 h after chlorination; B, 6 h after chlorination; C, 29 h after chlorination.

chlorine first appears ≈ 10 min after chlorine addition; the amount of Po decreases slowly to represent only 5% of the Po amount in the control cells, at the end of the

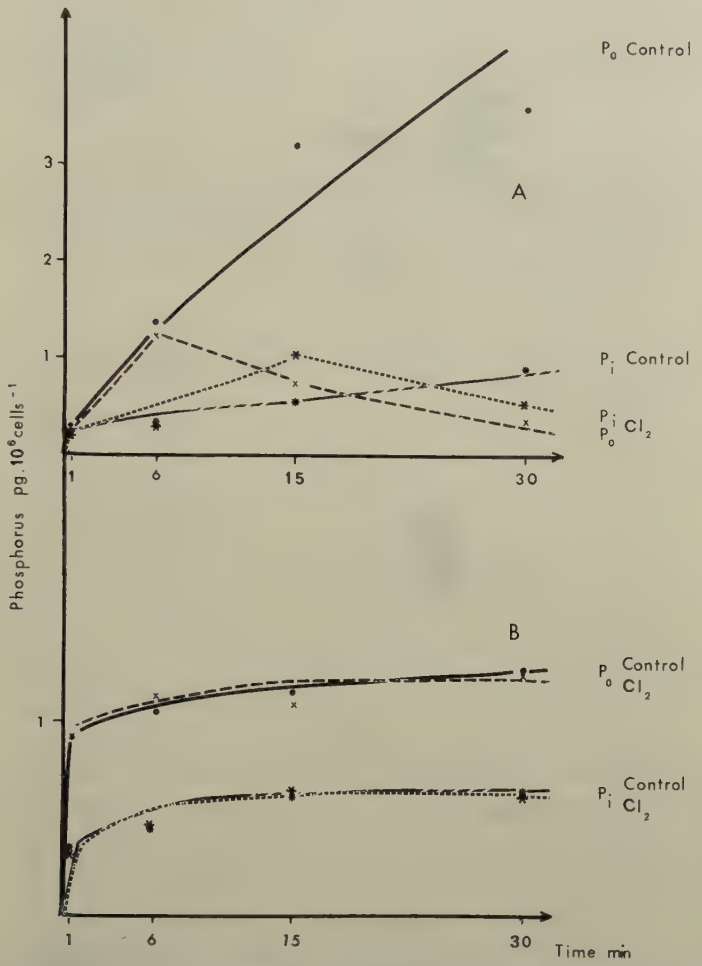


Fig. 4. Influence of chlorine on acid-soluble inorganic phosphorus (Pi) and organic phosphorus (Po) concentrations: A, immediately after chlorination; B, 24 h after chlorination (mortality = 50%).

absorption time. The effect of chlorine on the Pi amount appears later (≈15 min): the leakage noticed tends to prove that membrane permeability is affected, at least immediately after the chlorine shock stress. Indeed, 24 h after chlorination, the Pi and Po amounts in the surviving cells were identical to that of unchlorinated cells (Fig. 4B). The differences between Pi and Po kinetics are probably due to the inadequate growth in experimental conditions (filtered sea water only).

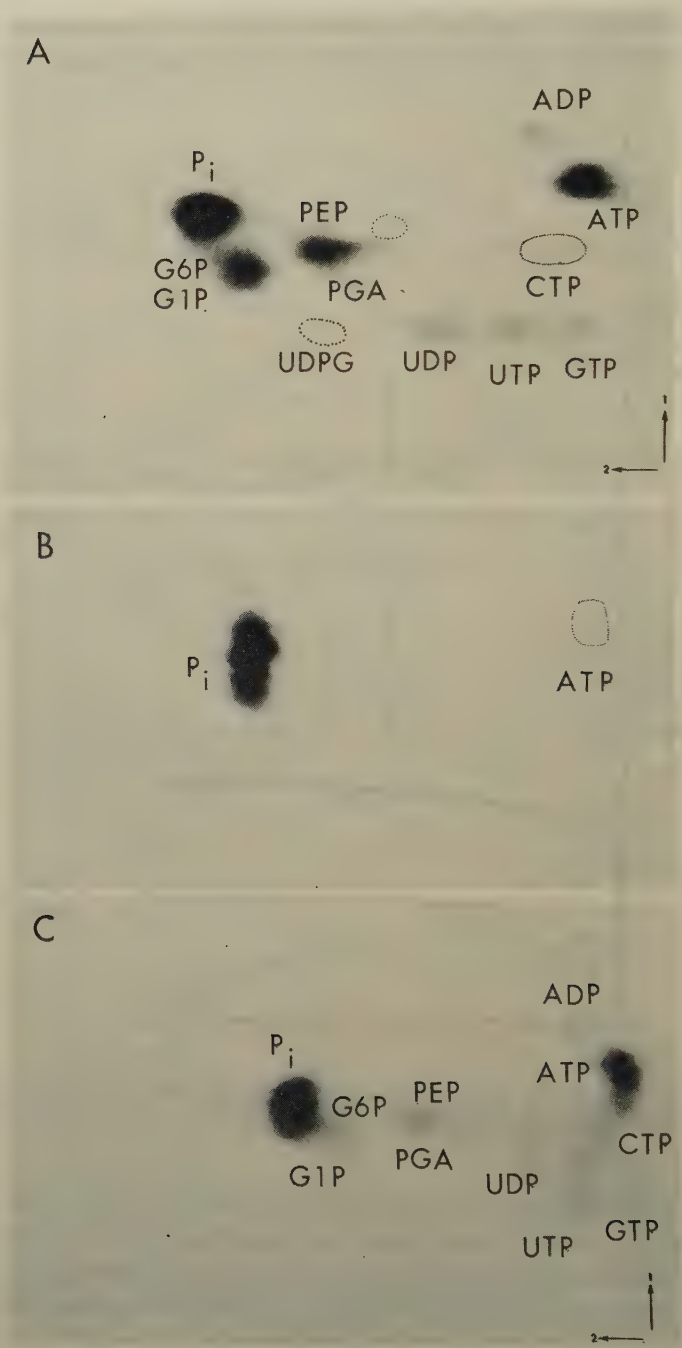


Fig. 5. Autoradiographs from phosphorylated compounds separated by two-dimensional chromatography after incubation with $^{32}PO_4^{3-}$ for 15 min: A, unchlorinated cells (control); B and C, chlorinated cells, 1 h (B) and 24 h (C) after chlorination.

*Phosphorylated compound analysis*

Chromatographic analysis was performed on chlorinated cells 1 h and 24 h after chlorination (0.6 ppm; 50% mortality) and 15 min incubation with $^{32}P$, and compared with a similar analysis of the control cells (under the same conditions). The results (Table IV, Fig. 5) show that among the various identified compounds in the control

TABLE IV

Incorporation of $^{32}P$ into organic compounds after incubation of *Dunaliella primolecta* with $^{32}PO_4^{3-}$ for 15 min: control = unchlorinated cells; $Cl_2$ = chlorinated cells after 1 h (mortality = 50%).

| Compounds | | % of total radioactivity | |
|---|---|---|---|
| | | Control | $Cl_2$ |
| Adenosine diphosphate | (ADP) | 5.8 | 0 |
| Adenosine triphosphate | (ATP) | 15.8 | 20 (30% of control) |
| Cytidine triphosphate | (CTP) | 4.7 | 0 |
| Guanosine triphosphate | (GTP) | 4.1 | 0 |
| Uridine triphosphate | (UTP) | 4.4 | 0 |
| Uridine diphosphate | (UDP) | 3.4 | 0 |
| Uridine diphosphate glucose | (UDPG) | 1.5 | 0 |
| Phosphoenolpyruvate | (PEP) | | |
| Phosphoglyceraldehyde | (PGA) | 9.5 | 0 |
| Glucose-1-phosphate | (G-1-P) | | |
| Glucose-6-phosphate | (G-6-P) | 13.5 | 0 |
| Unidentified | | 2.9 | 0 |
| Inorganic phosphate | Pi | 34 | 76 (20% of control) |

cells, ATP, hexose-phosphates (G-1-P, G-6-P), and Pi are predominant. The formation of phosphorylated compounds was completely inhibited, with the exception of ATP in the cells chlorinated 1 h before testing (Fig. 5B). While the amount of ATP corresponds to 20% of the total radioactivity, this amount is actually one-third of the ATP concentration in the control cells. Likewise, the absorbed Pi was only 20% of the Pi in the control cells. Twenty-four hours after chlorination, however, the chlorinated cell metabolism reverted to normal, as all phosphorylated compounds were traced (Fig. 5C).

*ATP content*

Among the various phosphorylated nucleotides, ATP is of particular interest because of its influence on enzyme control mechanisms or its use for biomass measurement. The analysis of phosphorylated compounds has shown that, although chlorine reduces the ATP content, it does not entirely inhibit its formation. The development of ATP in chlorinated cells was observed by means of the luciferin–luciferase procedure. The results (Fig. 6) corroborate two main aspects of chlorine

influence on the cells: (1) its fast action on metabolism – 80% of the ATP loss taking place within < 1 h, and (2) its short action – the surviving chlorinated cells

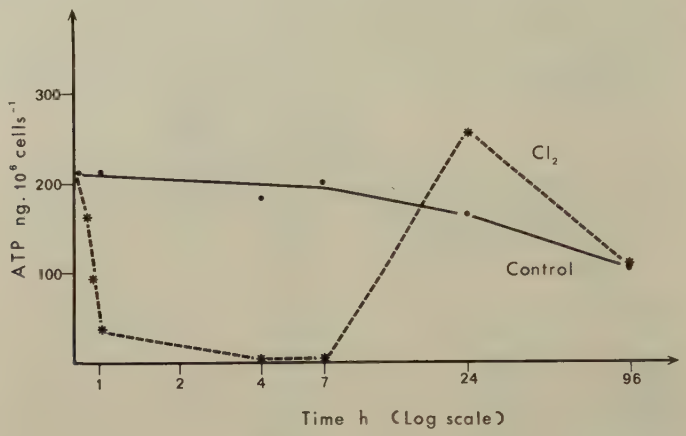


Fig. 6. Intracellular ATP contents in *Dunaliella* cells chlorinated ($Cl_2$) and unchlorinated (control) expressed as ng · $10^6$ cells$^{-1}$ (mortality = 90%).

recover very quickly (within <24 h) an ATP content at least equal to that of unchlorinated cells.

## Discussion

### CHLORINE ACTION ON OXYGEN EXCHANGES

Contrary to toxicity tests regarding the growth or pigment concentration of *D. primolecta*, recordings of the respiration and photosynthesis variations have shown that the action of chlorine on oxygen exchanges was immediately depressing. It lasts for a variable period according to the percentage chlorination but the further development of gas exchanges is difficult to explain, under the test conditions, owing to alterations appearing in the unchlorinated cells ≈16 h after the first measurements (data not shown). Those modifications may be due to cell density differences between chlorinated and unchlorinated cultures, to the nutrition-deficient medium (filtered sea water) or to oxygen concentration.

A different result of chlorination, related to light conditions, appears as regards respiration and photosynthesis. With an average or no mortality, chlorination performed in the light inhibits only photosynthesis (Table III). On the contrary, respiration is inhibited by chlorination in the dark, with no alternating light periods, and with a similar mortality being induced. With a higher mortality (>65%) and whatever the light conditions, both respiration and photosynthesis are inhibited, the latter being more affected. From these observations and from previous results,

regarding mortality and pigment concentration (Videau *et al.*, 1979), one could conceive a synergic action of the oxidizing agent and of light which not only would accelerate oxidation, but would first inhibit the photochemical process. Besides, it appears from the first metabolic test, that the effect of chlorine is not only immediate, but that it can reduce the photosynthetic process by 40% without causing any cell death or decreasing the growth rate.

In connection with phosphorylation processes dependent on photosynthesis and with membrane permeability, the investigation on phosphate absorption confirmed the high physiological susceptibility of *D. primolecta*.

## CHLORINE ACTION ON PHOSPHATE ION ABSORPTION AND METABOLISM

The penetration of large amounts of phosphate ions into the cells during the first 30 s of absorption is probably connected with the surface to volume ratio of the cell (Sutcliffe, 1962; Friebele *et al.*, 1978) and to the precariousness of ion bonds in the apparent free space (Gest & Kamen, 1948; Goldberg *et al.*, 1951). This is confirmed by the gradual development of total phosphorus fixed in the cell or by the ineffectual action of chlorine on absorption during those first 30 s.

On the other hand, chlorination even at a low concentration has a depressing effect on the second half of the phosphate uptake curve, even though there is no mortality or decline in growth. This absorption reduction is the result of two actions: (1) on metabolism: the low Po fraction of chlorinated cells shows the inhibition of the metabolic process: this inhibition appears before Pi decreases, and suggests a feedback action on phosphate absorption; and (2) on membrane permeability: the Pi amount decreases because of the inhibition of active transport, but there is also an ion leakage in the outer environment, which indicates a change in membrane permeability. It is also of interest to note that the rate of phosphate uptake is identical in surviving chlorinated cells and in unchlorinated cells a short time after heavy chlorination. The recovery of metabolic activity is confirmed by a similar Pi and Po fraction development, showing the absence of persisting membrane alteration or of metabolic decline in the surviving cells.

The analysis of phosphorylated compounds corroborates these conclusions and shows that the low Pi fraction incorporated in the cells, 1 h after chlorination represents only 20% of the amount in control cells. The organic fraction determined from Pi and Po separation, although small, corresponds entirely to the formation of ATP (30% of control cell ATP). This was checked by direct measurement of ATP. Accordingly, there is an inhibition of all phosphorylation processes, probably owing to transphosphorylase inactivation, as suggested by Venkobachar *et al.* (1977) in chlorinated bacteria. The absence of metabolic alteration in the surviving cells after the influence of chlorine disappeared 24 h after chlorination and all the organic compounds resulting from phosphate metabolism are present in the chlorinated as well as the unchlorinated cells.

To sum up, the oxidizing product (bromine in the present chlorination conditions), even in small amounts, induces an immediate metabolic inhibition, soon followed by an alteration of membrane permeability. The surviving cells, however, recover their full metabolism more or less quickly, according to the added amount of chlorine after the residual oxidizing power cannot be measured.

## References


Cole, C. V. & C. Ross, 1966. Extraction, separation and quantitative estimation of soluble nucleotides and sugar phosphates in plant tissues. *Analyt. Biochem.* Vol. 17, pp. 526–539.

Ducet, G. & G. Vandewalle, 1959. Nouvelles techniques du dosage du phosphore. *Ann. Phys. Veg.*, Vol. 2, pp. 199–206.

Eppley, R. W., E. H. Renger & P. M. Williams, 1976. Chlorine reactions with sea-water constituents and the inhibition of photosynthesis of natural marine phytoplankton. *Estuar. cstl mar. Sci.*, Vol. 4, pp. 147–151.

Floc'h, J. Y. & M. Penot, 1978. Changes in $^{32}$P-phosphorus compounds during translocation in *Laminaria digitata* (L.) Lamouroux. *Planta*, Vol. 143, pp. 101–107.

Friebele, E. S., D. L. Correll & M. A. Faust, 1978. Relationship between phytoplankton cell size and the rate of orthophosphate uptake; in situ observations of an estuarine population. *Mar. Biol.*, Vol. 45, pp. 39–52.

Gest, H. & M. D. Kamen, 1948. Studies on the phosphorus metabolism of green algae and purple bacteria in relation to photosynthesis. *J. biol. Chem.*, Vol. 176, pp. 299–318.

Goldberg, E. D., J. J. Walker & A. Whisenand, 1951. Phosphate utilization in diatoms. *Biol. Bull. mar. biol. Lab.; Woods Hole*, Vol. 101, pp. 274–284.

Holm-Hansen, O. & C. R. Booth, 1966. The measurement of adenosine triphosphate in the ocean and its ecological significance. *Limnol. Oceanogr.*, Vol. 11, pp. 510–519.

Inman, G. W. & J. D. Johnson, 1978. The effect of ammonia concentration on the chemistry of chlorinated seawater. Water Chlorination Environ. Impact Health. *Eff. Proc. Conf. 1977* (Pub. 1978), Vol. 18, pp. 235–252.

Jolley, R. L., R. B. Cumming & W. W. Pitt, 1978. Ecological impact of chloro-organics produced by chlorination of cooling tower waters. *Energy Research Abst.*, Vol. 3, p. 8 only.

Khalanski, M., 1977. Influence du fonctionnement d'une centrale thermique sur la production primaire planctonique du port de Dunkerque. *Rapport Electricité de France* (E.D.F.) E-311-77/n° 3, janv. 1977, 12 pp.

Kott, Y. & J. Edlis, 1969. Effect of halogens on algae: *Chlorella sorokiniana*. *Wat. Res.*, Vol. 3, pp. 251–256.

Peron, A. & J. Courtot, 1979. Étude des mécanismes réactionnels liés à l'injection de chlore dans l'eau de mer contenant de l'azote ammoniacal. *Rapport Electricité de France* (E.D.F.) E-33-78/017 ARD-E3 E01, 32 pp.

Sutcliffe, J. F., 1962. *Mineral salt absorption in plants*. Pergamon Press, Oxford, 194 pp.

Toetz, D., L. Varga & M. Pierce, 1977. Effects of chlorine on uptake of inorganic nitrogen by phytoplankton. *Wat. Res.*, Vol. 11, pp. 253–258.

Venkobachar, C., L. Lyengar & A. V. S. Prabhakara Rao, 1977. Mechanism of desinfection effect of chlorine on cell membrane functions. *Wat. Res.* Vol. 11, pp. 727–729.

Videau, C., M. Khalanski & M. Penot, 1979. Preliminary results concerning effects of chlorine on monospecific marine phytoplankton. *J. exp. mar. Biol. Ecol.*, Vol. 36, pp. 111–123.

Walne, P. R., 1966. Experiments in the large scale culture of the larvae of *Ostrea edulis* (L.). *Fishery Invest., Lond. (Ser. 2)*, Vol. 25, No. 4, 53 pp.

Wong, G. T. F. & J. A. Davidson, 1977. The fate of chlorine in seawater. A preliminary review. *Wat. Res.*, Vol. 11, pp. 971–978.

*J. exp. mar. Biol. Ecol.*, 1980, Vol. 47, pp.127–139
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# ASPECTS OF CARBON AND NITROGEN CYCLING IN A SHALLOW MARINE ENVIRONMENT

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**Abstract:** Studies on the water column of a semi-enclosed bay (Roskeeda Bay, Co. Galway) showed a close association between photosynthesis and respiration. Gross annual production of both the water column and the sediment was lower than the total annual decomposition. Sediment traps showed evidence of constant resuspension of fine sediment surface material, which frustrated the direct measurement of the transfer of particulate organic material from water to sediment. Fluxes of inorganic nitrogen compounds across the sediment water boundary were measured by using an in situ bell jar technique. Mean rates of release of nitrate and nitrite were insignificant compared with the release of ammonia, which was equivalent to 20% of the phytoplankton nitrogen requirement over the summer. An approximate diffusion coefficient of $4.5 \times 10^{-5}$ $cm^2 \cdot s^{-1}$ was estimated for ammonia release from the sediment. Ammonia release was strongly correlated with oxygen uptake by the sediment and less strongly with temperature. During the productive season nitrogen supply to the water column from the biological recycling of organic material in both water and sediment was in excess of phytoplankton requirements. As standing levels of inorganic nitrogen did not increase significantly over this period, it is suggested that the intertidal macrophyte community acts as both a sink of inorganic nitrogen and a source of organic carbon for the system. A tentative annual carbon budget is presented.

## INTRODUCTION

Light and the availability of inorganic nutrients are the main factors controlling marine productivity (Ryther, 1963). Unless controlled by physical processes, nutrient supply, and hence productivity, may be dependent on the biological decomposition of organic material. Nutrient regeneration is confined mainly to the near-surface layers of the ocean (Menzel, 1967; Menzel & Ryther, 1970). In shallow nearshore areas, however, this zone includes the benthos which may then become a significant source of inorganic nutrients.

In shallow areas, benthic nutrient regeneration has generally been studied by means of benthic enclosures. Davies (1975) investigated regeneration by the muddy sediments of a Scottish sea loch and found that ammonia released from the sediment was sufficient to provide > 50% of the nitrogen required by the overlying phytoplankton. Hartwig (1976) and Rowe *et al.* (1977) studied regeneration by coastal siliceous sediments. Rowe *et al.* estimated that 30–40% of the nitrogen required by the phytoplankton was supplied by sediments off the Spanish Sahara, but Hartwig working off the Californian coast stated that the release of inorganic nutrients by the sediment made no significant contribution to levels in the overlying water.

Estimations of benthic nutrient release have also been made based on seasonal changes and gradients of nutrients within sediments (Rittenberg *et al.*, 1955; Thorstensen & MacKenzie, 1974) and on concentration gradients in the water column. These estimates rely, however, on several assumptions. By studying gradients in the water column, Rowe *et al.* (1975) were able to show that benthic nutrient regeneration contributed significantly to the supply of nitrogen (as ammonia) in continental shelf waters off New York. In several studies, attempts have been made to estimate the fraction of the nutrient requirement of overlying phytoplankton which is supplied by release from the sediment. With the exception of Davies (1975) these estimates were based on previously published data on the primary productivity of the area studied from which nutrient requirements were calculated using the ratios of Redfield *et al.* (1963). In no case was the biological recycling of nutrients estimated for the water column. The necessity of including this factor in studies on the supply of inorganic nutrients is illustrated by the work of Smith & Whitledge (1977) who estimated that, in nutrient-rich upwelling waters off northwestern Africa, $\approx 75\%$ of primary production is supported by nitrogen regenerated by pelagic animal populations.

As part of a research programme investigating the production of a coastal inlet off the west coast of Ireland, an attempt was made to clarify the relative importance of the sediment and water systems as sites of both oxidation of organic material and nutrient regeneration. Some results of this study are presented here, together with observations of the mechanisms involved in the coupling of carbon and nutrient flux between the benthos and water column.

## Materials and Methods

The work was carried out in Roskeeda Bay (Inner), Co. Galway, Eire, (53°21′10″ N : 9°40′50″W), a semi-enclosed inlet of $\approx 1$ km$^2$ with an average depth of 8 m, although depths up to 22 m are encountered. Primary production was measured using the oxygen technique, the titration procedure used being the precise Winkler modification of Bryan *et al.* (1976). Light and dark bottles were exposed in situ at least in triplicate at 0, 2, 5, and 10 m for 24 h. The methods used for the analysis of pigments and inorganic nutrients (nitrate, nitrite, and phosphate) were those described by Strickland & Parsons (1972). Ammonia was analysed using the method of Koroleff as outlined by Riley *et al.* (1972).

The flux of nutrients across the water–sediment interface was measured by a bell-jar technique (Raine & Patching, 1979). Water for nutrient analysis was sub-sampled from samples for oxygen determinations and analysed by the aforementioned techniques suitably modified for the smaller sample volume (10 ml). Sedimentation of particulate material was monitored by means of a sedimentation trap located in the study area (Raine, 1979). Extraction of sediment interstitial

water for ammonia analysis was made from cores collected by divers (Raine & Patching, 1979). Sections (2 cm) of sediment core were centrifuged and the supernatant filtered (Whatman GF/C). This procedure was carried out as rapidly as possible and within 2 h of sampling. Analysis was done immediately on the extracts.

## Results

To assess the annual cycling of carbon in the water column, measurement of primary production and heterotrophic activity, as estimated by dark bottle oxygen consumption, were made regularly throughout 1977, together with standing stock estimates of chlorophyll *a* concentration (Fig. 1.). At no stage in the year was there any evidence of stratification within the Bay, the difference in temperature between surface and bottom water never being in excess of 0.2 °C. Seasonal variations in temperature together with changes in inorganic nutrient concentrations in the water column are shown in Fig. 2.

The principal form of inorganic combined nitrogen released by the sediment was ammonia (Fig. 3.). The overall release of nitrate was small and there was no significant release of nitrite, suggesting that nitrification in the sediment was not a major influence on the nutrient cycling processes of the Bay. The peak of ammonia release in August correlated with a peak in ammonia concentration in the water column, and may have supplied the nutrient requirement for the algal bloom observed in late August. Use of transparent or opaque bell jars caused no significant difference in the rates of flux of nutrients.

Linear regression coefficients for release of ammonia and oxygen uptake by the sediment and temperature are shown in Table I. A strong relationship was found between the release of ammonia and oxygen uptake. Multiple linear regression analysis showed that temperature was not significant relative to oxygen uptake in explaining the variability in results of ammonia release. Table II summarizes the ammonia concentrations in the interstitial water in the top 2 cm of the sediment and ammonia concentrations in the water column immediately overlying the sediment, as well as the observed rates of ammonia release. Values of ammonia concentrations in the water column are measurements taken from initial samples of the bell-jar experiments. These may be overestimates; physical emplacement of the bell-jars would disturb the sediment system, thereby releasing ammonia, which may not have been completely flushed out during the equilibration period. Using these figures it was possible to calculate observed diffusion coefficients for ammonia released into the water column using the equation:

$$\text{ammonia flux} = \frac{K' \cdot [(NH_3)s - (NH_3)w]}{z},$$

where $(NH_3)s$ is the ammonia concentration in the top 2 cm of sediment, $(NH_3)w$ is the ammonia concentration in the water column overlying the sediment, $z$ is the

distance, and $K'$ is the observed diffusion coefficient. It was assumed that a linear gradient in ammonia concentration over the top 2 cm existed. Thus, values of $(NH_3)s$ represented the concentration at 1 cm depth, and this was taken as the value of $z$. This type of gradient may not have existed, but the data obtained were insufficient to determine a more accurate estimate. Calculated values of $K'$ are listed in Table II. The mean of these values was $4.5 \times 10^{-5}$ cm$^2 \cdot$ s$^{-1}$ (range $1.65 - 8.6 \times 10^{-5}$ cm$^2 \cdot$ s$^{-1}$; SD $= 2.3 \times 10^{-5}$ cm$^2 \cdot$ s$^{-1}$). The major error in this calculation is in the estimate of $(NH_3)s$ ($\gg (NH_3)w$).

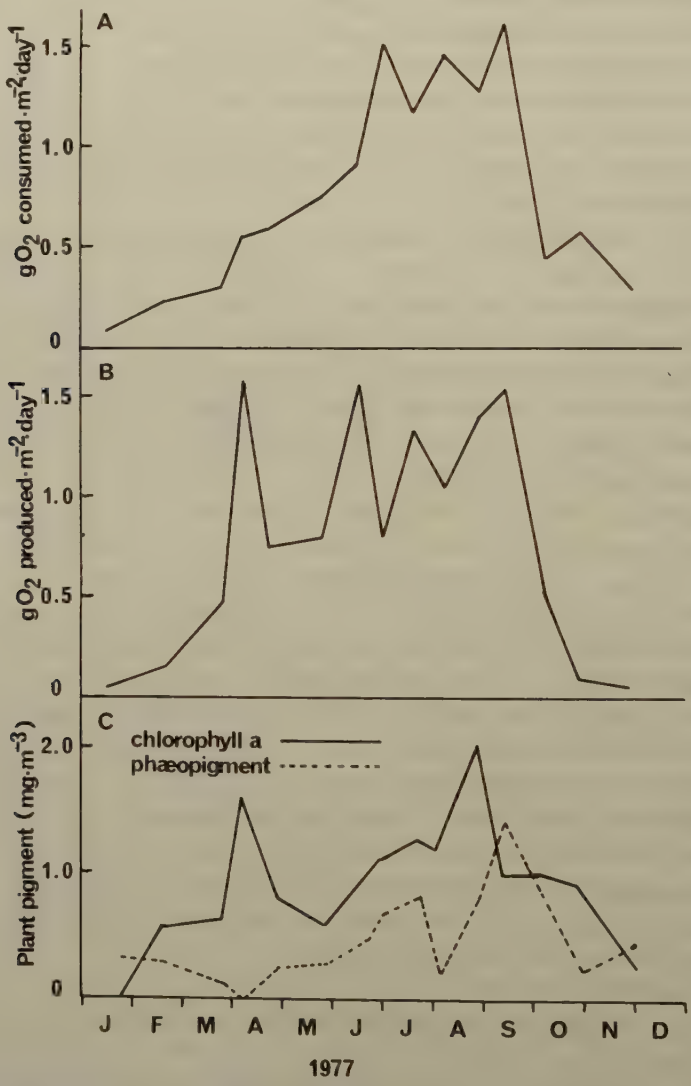


Fig. 1. Seasonal variations of heterotrophic activity (A), gross primary production (B), and surface chlorophyll and phaeopigment (C) in the water column.

Sediment traps positioned in the Bay were found consistently to collect a mixture of resuspended and de novo sedimenting material. The presence of the former (identified by the presence of healthy benthic diatom populations, sponge spicules and dead seaweed fragments) permitted only qualitative observations on sedimenting material. About 20% (by volume) of trapped material could be readily identified

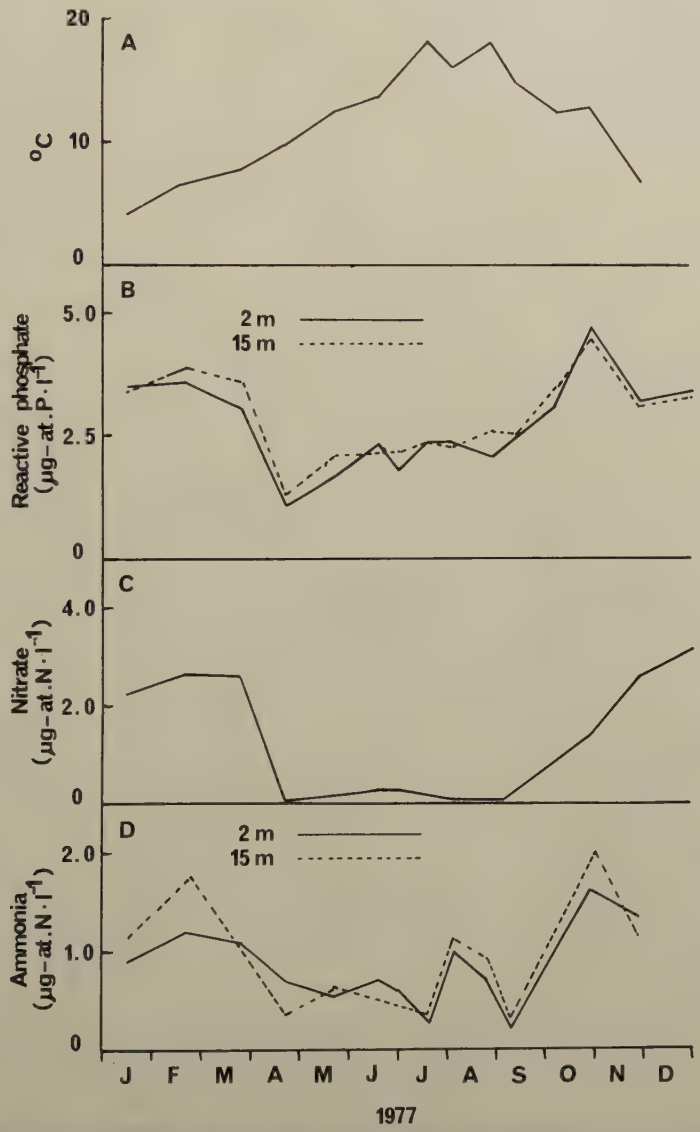


Fig. 2. Seasonal variations of mean temperature (A), phosphate (B), nitrate (C), and ammonia (D) in the water column: nitrate concentrations are the mean of 2-m and 15-m samples, the difference between these never exceeded 0.1 $\mu$g-at. $N \cdot l^{-1}$.

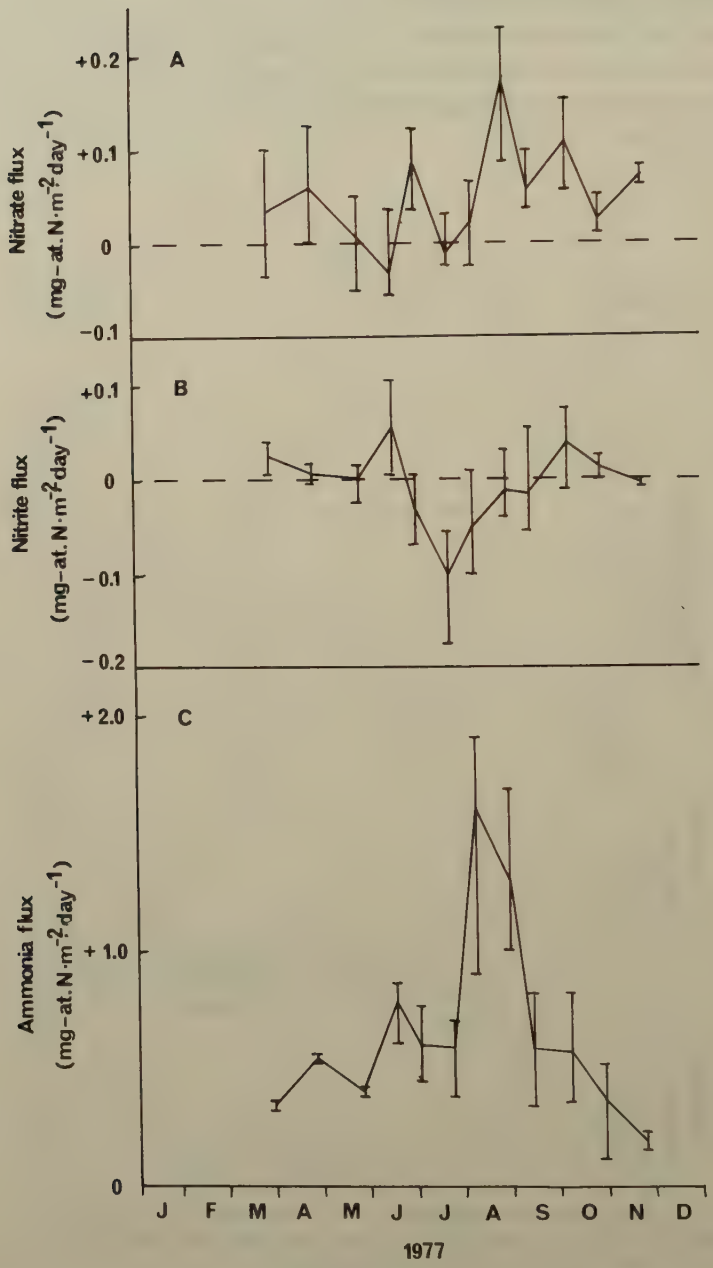


Fig. 3. Exchange of nitrate (A), nitrite (B), and ammonia (C) between sediment and water column.

and of this the major fraction consisted of intact zooplankton faecal pellets. This is in accordance with the findings of other workers (cited in Hargrave & Taguchi, 1978).

TABLE I

Slope and intercept coefficients of regression analysis of temperature and oxygen uptake with ammonia release by the sediment: figures in parentheses represent SE of the slope and intercept.

| | Slope | Intercept | $r$ | $n$ |
|---|---|---|---|---|
| Temperature (°C) with oxygen uptake ($mg\text{-}at.\ O \cdot m^{-2} \cdot day^{-1}$) | 2.3 (0.5) | −8.1 (7.0) | 0.69 | 23 |
| ammonia release ($mg\text{-}at.\ N \cdot m^{-2} \cdot day^{-1}$) | 0.08 (0.02) | −0.3 (0.3) | 0.62 | 23 |
| Oxygen consumption ($mg\text{-}at.\ O \cdot m^{-2} \cdot day^{-1}$) with ammonia release ($mg\text{-}at.\ N \cdot m^{-2} \cdot day^{-1}$) | 0.035 (0.003) | −0.06 (0.08) | 0.92 | 23 |

TABLE II

Calculated values of observed diffusion coefficients ($K'$) of ammonia through the sediment–water interface, Roskeeda Bay: a summary of the data from which these values are calculated is also shown; a, mean of results from two cores – figures in parentheses denote observed variation; b, mean of four observations (except 25 March when only two observations were made) – figures in parentheses represent the SD (except for 25 March when observed variation on the mean of two measurements is shown).

| Date 1977 | Concentration of ammonia in interstitial water of top 2 cm of sediment[a] ($\mu g\text{-}at.\ N \cdot l^{-1}$) | Initial ammonia concentration in bell-jars[b] ($\mu g\text{-}at.\ N \cdot l^{-1}$) | Measured rate of diffusion of ammonia out of the sediment[b] ($mg\text{-}at.\ N \cdot m^{-2} \cdot day$) | $K'$ ($cm^2 \cdot s^{-1}$) |
|---|---|---|---|---|
| 25 Mar. | 10 (±3) | 2.1 (0.4) | 0.32 (0.02) | $4.6 \times 10^{-5}$ |
| 20 Apr. | 15 (±3) | 2.2 (0.5) | 0.55 (0.03) | $6.5 \times 10^{-5}$ |
| 21 May | 18 (±5) | 2.0 (0.5) | 0.38 (0.03) | $3.4 \times 10^{-5}$ |
| 18 June | 19 (±4) | 1.1 (0.2) | 0.77 (0.12) | $6.4 \times 10^{-5}$ |
| 18 July | 18 (±3) | 0.7 (0.2) | 0.59 (0.15) | $3.5 \times 10^{-5}$ |
| 5 Aug. | 23 (±2) | 1.5 (0.4) | 1.59 (0.52) | $8.6 \times 10^{-5}$ |
| 25 Aug. | 25 (±4) | 1.4 (0.6) | 1.27 (0.29) | $6.2 \times 10^{-5}$ |
| 9 Sept. | 25 (±6) | 0.8 (0.3) | 0.58 (0.26) | $2.8 \times 10^{-5}$ |
| 7 Oct. | 21 (±3) | 1.0 (0.4) | 0.57 (0.25) | $3.3 \times 10^{-5}$ |
| 25 Oct. | 14 (±2) | 2.2 (0.7) | 0.36 (0.18) | $3.5 \times 10^{-5}$ |
| 25 Nov. | 18 (±2) | 3.4 (1.2) | 0.20 (0.04) | $1.7 \times 10^{-5}$ |

Another route supplying organic material to the benthos was identified in October when fresh seaweed fragments with intact plastids were found in the traps. These fragments had presumably been broken free during a period of storms in that month. A proportion of identifiable material in sediment traps consisted of faecal pellets in various stages of decomposition. These presumably had their origins as resuspended

material. Mineral grains were rarely evident, suggesting that sediment resuspension was, in the main, confined to the fine easily disturbed surface layers of the sediment.

## Discussion

The cycle of primary production and the consequences this has on the inorganic nutrient concentrations found in Roskeeda Bay follow a well-established pattern for such processes in temperate regions. One point of note is the marked seasonal variation in heterotrophic activity, as measured by dark bottle oxygen uptake, and its close association with primary production. Such close associations could be due to an absence of major external sources of organic material. This conclusion is supported by the data of Souza Lima & Williams (1978) who studied planktonic oxygen consumption in Southampton Water, England. A comparison of the results from two stations in the Estuary, one receiving external additions of organic material and one further seaward, shows more marked seasonal variation in oxygen consumption in the latter case than in the former. Souza Lima & Williams (1978) did not find as good an association between respiration and photosynthesis as either Williams (1966) or that observed in this study. A low level of winter respiration is evident in the data of Williams (1966) and winter rates observed in Roskeeda Bay were at the limit of detection of the analytical method used. Souza Lima & Williams (1978), however, found relatively high rates of oxygen consumption (200 $\mu$g $O_2 \cdot l^{-1} \cdot day^{-1}$) in winter for a station near to an external input of organic material (see Souza Lima & Williams, 1978; Station 3, Fig. 1). Annual primary production and decomposition in the water column of Roskeeda Bay were equivalent to 245 g oxygen produced $\cdot m^{-2}$, and 228 g oxygen consumed $\cdot m^{-2}$, respectively. Use of a photosynthetic quotient of 1.2 (Strickland, 1960) provides a figure for gross annual production of 73.5 g carbon $\cdot m^{-2}$. Higher values of photosynthetic quotient have been observed (Williams *et al.*, 1979) but conditions leading to these (high nitrate concentrations) were not encountered during this study. Use of an oxygen utilization: carbon remineralization ratio of 276 : 106 (Redfield *et al.*, 1963) provides a figure for annual decomposition of 69.3 g carbon $\cdot m^{-2}$.

In shallow inshore areas a considerable proportion of organic matter produced in the water column may settle to decompose or accumulate in the sediment. Measurements of benthic respiration and productivity carried out during the course of this study (Raine & Patching, 1979) show an annual requirement for at least 20 g exogenous carbon $\cdot m^{-2}$. This is equivalent to 27% of the annual primary production of the water column. Davies (1975) demonstrated that 30% of the annual organic carbon production of a shallow Scottish sea loch settled to the sediment.

Hargrave (1973) has derived an expression equating carbon supply from the water column to its utilization by bottom communities. The data presented here fall near but below the regression line of the logarithmic plot of primary production/

mixed layer depth against sediment oxygen uptake, which he prepared from the data of several workers. The apparently decreased level of sediment oxygen uptake may be due to recently sedimented material being resuspended into the water column and decomposing there. This is supported by the consistent finding of resuspended light sediment–surface material in sediment traps. Resuspension frustrated the use of sediment traps to measure directly the supply of particulate organic matter from the water column to the sediment. It would appear, however, that the gross phytoplankton production of organic matter was insufficient to supply that decomposed by both sediment and water column.

The decomposition of organic matter in marine sediments will produce inorganic nutrients which may eventually be released to the overlying water. The relative rates of inorganic combined nitrogen flux across the sediment–water boundary show the flux of ammonia to dominate over that of nitrate and nitrite. This is in agreement with the observations of Davies (1975), Hartwig (1976), and Nixon *et al.* (1976). The atomic organic C : N ratio of the sediment was 10.6 : 1, and the observed atomic ratio for rates of oxygen consumption and release of ammonia by the sediment was 28.6 : 1. If the oxidation of organic material were the prime factor controlling the liberation of ammonia to the water column, then one could extrapolate these ratios further to obtain an O : C ratio of 2.7 : 1 (28.6 : 10.6). This ratio represents an equivalent respiratory quotient of 0.74 which is within the range observed for marine sediments by Teal & Kanwisher (1961) of 0.55–0.96. This approach, however, would appear to be too simplistic, as any preferential release of nitrogen over carbon would affect this type of calculation.

Another major factor that may ultimately control the liberation of ammonia to the water column is diffusion. The concentration gradient of ammonia across the sediment–water interface will depend essentially on its concentration in the interstitial water, which is far in excess of that in the overlying water, and the concentration in the interstitial water will depend on organic nitrogen metabolism within the sediment, adsorption onto sediment particles and loss by diffusion into the water column. Biological organic nitrogen metabolism will be a function of temperature and the supply of organic material, which itself may be a function of temperature. It is, therefore, not surprising that a significant relationship was found between rates of ammonia release and temperature. Diffusion coefficients are also affected by temperature. A major criticism of the calculation used to obtain values for $K'$ (the observed diffusion coefficient) is that each estimate was derived from only two measurements of concentration. This of necessity implies linearity between the points, which probably did not exist, and the mean $K'$ ($4.5 \times 10^{-5}$ cm$^2 \cdot$ s$^{-1}$) can only be treated as a rough value; furthermore, the compounded errors in the calculation of $K'$ were large, and hence no significant variation of $K'$ with temperature or season was apparent (Table II). Estimates, however, compare favourably with values of $1 \times 10^{-6}$ cm$^2 \cdot$ s$^{-1}$ (Berner, 1974) and $1–5 \times 10^{-5}$ cm$^2 \cdot$ s$^{-1}$ (Vanderborght & Billen, 1975) quoted in the literature, which suggests that the ultimate process

returning ammonia to the water column from the sediment was diffusion. The net effect of both increased biological activity and temperature is to increase the rates of diffusion of both ammonia out of, and oxygen into, the sediment. Biological activity is itself correlated positively with temperature, but may also be affected by other factors e.g., the supply of organic material. It might be expected, therefore, that any relationship between oxygen uptake and release of ammonia would be better than the relationship of either of these processes with temperature. This was the case for this study. Although the observations of both Davies (1975) and Hartwig (1976, 1978) suggest that processes controlling the return of carbon and nitrogen to the water column are not the same, Rowe and his co-workers (Rowe *et al.*, 1975, 1977; Rowe & Smith, 1977) have estimated inorganic nitrogen release from measurements of oxygen uptake by the sediment. Use of an assumed respiratory quotient and the organic C : N ratio of the sediment enabled stoichiometric conversion of oxygen consumption into theoretical release of inorganic nitrogen compounds. Good agreement was found between the theoretical values and those measured in situ. The validity of using a similar assumed stoichiometry may be questioned for the sediment in Roskeeda Bay. The strong relationship between oxygen uptake and release of ammonia already discussed might suggest that routes of inorganic carbon and nitrogen return are the same. The resulting apparent respiratory quotient of 0.74 is the same as, or near to, many that have been quoted in the literature (Hargrave, 1973; Davies, 1975; Rowe *et al.*, 1975, 1977). It is difficult to decide whether this is fortuitous, that is to say the diffusion-controlled processes for ammonia and oxygen transfer happen to be in the same proportion as their biological release and uptake, or whether this is a real reflection of the biologically controlled chemical dynamics occurring within the sediment. If the processes were solely diffusion-controlled, then one would expect long-term changes in the organic carbon and nitrogen content of the sediment. In this instance, diffusion of the materials would be the rate-limiting step. Such long-term changes in organic carbon content were not observed (Raine & Patching, 1979). The analytical technique used, however, lacks the sensitivity required to observe such long-term changes over the high organic carbon background ($\approx 6.2\%$).

The importance of the benthos as a site of nutrient regeneration for maintaining the overlying production of Roskeeda Bay may now be qualified. The phytoplankton community is dependent on recycling of nitrogen in the 200-day period between May and October (Fig. 2). Over this period, production was 55.5 g carbon $\cdot$ m$^{-2}$ and the supply of ammonia from the sediment was 2.0 g nitrogen $\cdot$ m$^{-2}$. It is likely that the latter figure is an under-estimate since the technique used by us would not estimate nutrient release caused by wave-induced resuspension of sediments (Davies, 1975; Hartwig, 1976). The assimilation ratio of carbon : nitrogen by phytoplankton may have a range of 4 to 15, depending on the physiological state of the algal cells (see Williams *et al.*, 1979). Phytoplankton C : N ratios could not be measured in this study due to the high detrital concentrations experienced (Raine, 1979). Assuming

a mean C : N composition of plankton of 6.6 (Redfield *et al.*, 1963) the benthos supplied sufficient nitrogen to satisfy ≈20% of the nitrogen required by the phytoplankton. This figure can be compared with the findings of Davies (1975) who estimated that over half the nitrogen requirement of the phytoplankton could be supplied by the benthos in a similar environment. Over the productive season (200 days) heterotrophic activity in the water column amounted to 58.5 g carbon decomposed · $m^{-2}$. According to the ratios of Redfield *et al.* (1963), this should produce 10.3 g nitrogen · $m^{-2}$. This alone is sufficient to satisfy the nitrogen requirement of the phytoplankton over this period. Thus, since input of ammonia to the water column from the biological recycling of organic material in both water and sediment is greater than that required by the phytoplankton, one might expect a gradual increase in total inorganic nitrogen concentrations over the summer.

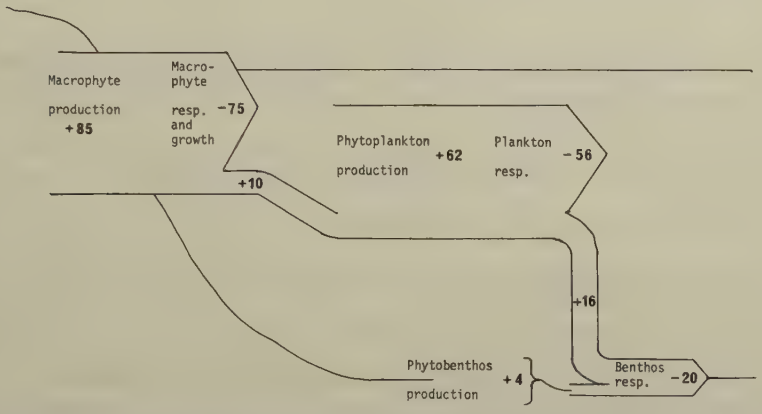


Fig. 4. Summary of proposed annual organic carbon budget for Roskeeda Bay, 1977: units represent tonnes of organic carbon produced (+) or consumed (−); resp., respiration.

The results presented here appear to indicate a further source of organic carbon and sink of inorganic nitrogen, not measured by us, in Roskeeda Bay. Both these rôles may be assumed by the macrophyte community of *Ascophyllum nodosum* (L.) Le Jol and *Fucus vesiculosus* (L.) which is found in the rocky intertidal zone of the Bay. The gross productivity of this community has recently been established as ≈85 tonnes carbon · $yr^{-1}$ (D. MacLoughlin, pers. comm.). The apparent difference between our measured values of overall production and decomposition in Roskeeda Bay is 10 tonnes carbon · $yr^{-1}$. Thus a balance between these processes could be achieved if ≈12% of the annual macrophyte production of organic carbon entered the rest of the system. Up to 40% of macrophyte production may be as dissolved organic material (Khailov & Burlakova, 1969; Sieburth, 1969). Webster *et al.* (1975) have suggested that 10–15% of macrophyte production reached the sediment as detrital seaweed fragments in St. Margaret's Bay, Nova Scotia. Thus, the macrophyte community is capable of supplying organic carbon in excess of that required

to make good the deficit in our proposed carbon budget. The excess may be decomposed within the macrophyte community. Alternatively, sediment oxygen consumption may provide an under-estimate of organic carbon breakdown (Pamatmat, 1977). Fig. 4 provides a tentative summary of organic carbon flow processes occurring in Roskeeda Bay 1977.

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## References

Berner, R. A., 1974. Kinetic models for the early diagenesis of nitrogen, sulfur, phosphorus and silicon in anoxic marine sediments. In, *The sea, Vol. 5*, edited by E. D. Goldberg, Wiley-Interscience, New York, pp. 427–450.

Bryan, J. R., J. P. Riley & P. J. LeB. Williams, 1976. A Winkler procedure for making precise measurements of oxygen concentration for productivity and related studies. *J. exp. mar. Biol. Ecol.*, Vol. 21, pp. 191–197.

Davies, J. M., 1975. Energy flow through the benthos in a Scottish sea loch. *Mar. Biol.*, Vol. 31, pp. 353–362.

Hargrave, B. T., 1973. Coupling carbon flow through some pelagic and benthic communities. *J. Fish. Res. Bd Can.*, Vol. 30, pp. 1317–1326.

Hargrave, B. T. & S. Taguchi, 1978. Origin of deposited material sedimented in a marine bay. *J. Fish. Res. Bd Can.*, Vol. 35, pp. 1604–1613.

Hartwig, E. O., 1976. The impact of nitrogen and phosphorus release from a siliceous sediment on the overlying water. In, *Estuarine processes, Vol. 1*, edited by M. Wiley, Academic Press, New York, pp. 103–117.

Hartwig, E. O., 1978. Factors affecting respiration and photosynthesis by the benthic community of a subtidal siliceous sediment. *Mar. Biol.*, Vol. 46, pp. 283–293.

Khailov, K. M. & Z. P. Burlakova, 1969. Release of dissolved organic matter by marine seaweeds and distribution of their total organic matter by marine seaweeds and distribution of their total organic production to inshore communities. *Limnol. Oceanogr.*, Vol. 14, pp. 521–527.

Menzel, D. W., 1967. Particulate organic carbon in the deep-sea. *Deep-Sea Res.*, Vol. 14, pp. 229–238.

Menzel, D. W. & J. H. Ryther, 1970. Distribution and cycling of organic matter in the oceans. In, *Organic matter in natural waters*, edited by D. W. Hood, Inst. Mar. Sci. (Alaska) Occas. Pub., Vol. 1, University of Alaska, pp. 31–54.

Nixon, S. W., C. A. Oviatt & S. S. Hale, 1976. Nitrogen regeneration and the metabolism of coastal marine bottom communities. In, *The role of terrestrial and aquatic organisms in decomposition processes*, Symp. Brit. ecol. Soc., Vol. 17, pp. 269–283.

Pamatmat, M. M., 1977. Benthic community metabolism: a review and assessment of present status and outlook. In, *Ecology of the marine benthos*, edited by B. E. Coull, Belle W. Baruch Library in Marine Science, Vol. 6, University of South Carolina Press, Columbia, pp. 89–111.

Raine, R. C. T., 1979. The influence of the benthos on the energy budget of a semi-enclosed bay. Ph.D. thesis, National University of Ireland, Dublin, 186 pp.

Raine, R. C. T. & J. W. Patching, 1979. Seasonal cycles of decomposition and production of organic material by the benthos of a semi-enclosed bay. In, *Cyclic phenomena in marine plants and animals, Proc. 13th Europ. mar. biol. Symp.*, edited by E. Naylor & R. G. Hartnoll, Pergamon Press, Oxford, pp. 241–248.

REDFIELD, A. C., B. H. KETCHUM & F. A. RICHARDS, 1963. The influence of organisms on the composition of sea water. In, *The sea, Vol. 2*, edited by M. N. Hill, Wiley-Interscience, New York, pp. 26–77.

RILEY, J. P., K. GRASSHOFF & A. VOIPIO, 1972. Nutrient chemicals. In, *A guide to marine pollution*, edited by E. D. Goldberg, Gordon and Breach, London, pp. 81–110.

RITTENBERG, S., K. O. EMERY & W. L. ORR, 1955. Regeneration of nutrients in sediments of marine basins. *Deep-Sea Res.*, Vol. 3, pp. 214–228.

ROWE, G. T., C. H. CLIFFORD & K. L. SMITH, 1977. Nutrient regeneration in sediments off Cap Blanc, Spanish Sahara. *Deep-Sea Res.*, Vol. 24, pp. 57–63.

ROWE, G. T., C. H. CLIFFORD, K. L. SMITH & P. L. HAMILTON, 1975. Benthic nutrient regeneration and its coupling to primary productivity in coastal waters. *Nature, Lond.*, Vol. 255, pp. 215–216.

ROWE, G. T. & K. L. SMITH, 1977. Benthic-pelagic coupling in the Mid-Atlantic Bight. In, *Ecology of the marine benthos*, edited by B. E. Coull, Belle W. Baruch Library in Marine Science, Vol. 6, University of South Carolina Press, Columbia, pp. 55–65.

RYTHER, J. H., 1963. Geographic variations in productivity. In, *The sea, Vol. 2*, edited by M. N. Hill, Wiley-Interscience, New York, pp. 347–380.

SIEBURTH, J. MCN., 1969. Studies on algal substances in the sea. III. The production of extracellular organic matter by littoral marine algae. *J. exp. mar. Biol. Ecol.*, Vol. 3, pp. 290–301.

SMITH, S. L. & T. E. WHITLEDGE, 1977. The role of zooplankton in the regeneration of nitrogen in a coastal upwelling system off northwest Africa. *Deep-Sea Res.*, Vol. 24, pp. 49–56.

SOUSA LIMA, H. DE & P. J. LEB. WILLIAMS, 1978. Oxygen consumption by the planktonic population of an estuary – Southampton water. *Estuar. cstl mar. Sci.*, Vol. 6, pp. 515–521.

STRICKLAND, J. D. H., 1960. Measuring the production of marine phytoplankton. *Bull. Fish. Res. Bd Can.*, No. 122, 172 pp.

STRICKLAND, J. D. H. & T. R. PARSONS, 1972. A practical handbook of seawater analysis. *Bull. Fish. Res. Bd Can.*, No. 167, 2nd edition, 310 pp.

TEAL, J. M. & J. KANWISHER, 1961. Gas exchange in a Georgia salt marsh. *Limnol. Oceanogr.*, Vol. 6, pp. 388–399.

THORSTENSEN, D. C. & F. T. MACKENZIE, 1974. Time variability of pore water chemistry in recent carbonate sediments, Devil's Hole, Harrington Sound, Bermuda. *Geochim. Cosmochim. Acta*, Vol. 38, pp. 1–19.

VANDERBORGHT, J.-P. & G. BILLEN, 1975. Vertical distribution of nitrate concentration in interstitial water of marine sediments with nitrification and denitrification. *Limnol. Oceanogr.*, Vol. 20, pp. 953–961.

WEBSTER, T. J., M. A. PARANJAPE & K. H. MANN, 1975. Sedimentation of organic matter in St. Margaret's Bay, Nova Scotia. *J. Fish. Res. Bd Can.*, Vol. 32, pp. 1399–1407.

WILLIAMS, P. J. LEB., R. C. T. RAINE & J. R. BRYAN, 1979. Agreement between the 14C and oxygen methods of measuring phytoplankton production: a reassessment of the photosynthetic quotient of marine plankton. *Oceanologica Acta*, Vol. 2, pp. 411–416.

WILLIAMS, R. B., 1966. Annual phytoplankton production in a system of shallow temperate estuaries. In, *Some contemporary studies in marine science*, edited by H. Barnes, George Allen & Unwin Ltd., London, pp. 699–716.

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# TEMPERATURE AND SALINITY EFFECTS ON THE RESPIRATORY METABOLISM OF THE FIRST ZOEAL STAGE OF *MACROBRACHIUM HOLTHUISI* Genofre & Lobão (DECAPODA: PALAEMONIDAE)

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**Abstract:** Oxygen consumption rates of stage I *Macrobrachium holthuisi* Genofre & Lobão zoeae were measured in 24 different temperature and salinity combinations using Cartesian diver microrespirometers. Metabolic rates varied little with salinity at 15 °C while at 20 °C a marked elevation occurred in 0 and 35‰. At 25 °C, a slight elevation occurred in 0‰; rates remained constant, however, in the other salinities. At 30 °C, respiratory rates were similar to those recorded at 25 °C except for decreases at 0 and 28‰ salinity. $Q_{10}$ values in the different salinities were usually highest between 15 and 20 °C. Statistical analyses showed that while both temperature, salinity and their interaction significantly influenced larval respiratory rates, temperature had the more pronounced effect. Larval metabolism is salinity independent over the salinity range encountered in the larval biotope (7–21‰) at temperatures of 15–30 °C.

## INTRODUCTION

Ovigerous females of the genus *Macrobrachium* habitually migrate to estuarine waters to spawn (Choudhury, 1970, 1971; Monaco, 1975; Nelson *et al.*, 1977; Moreira *et al.*, 1979). Populations of *Macrobrachium holthuisi* Genofre & Lobão from the São Paulo coast in southern Brazil characteristically release their eggs in waters of 20–30 °C and 0–14‰ salinity, typical of the small estuaries in which this decapod can be found. The resulting planktotrophic larvae develop over several months in the outer estuary before migrating to freshwater regions as juveniles. Larval tolerances to temperature and salinity variations for this species have already been assessed in the laboratory, optima were recorded at 20–25 °C and 14‰ salinity (Moreira *et al.*, 1979).

Although the effects of temperature and salinity on larval survival have been well documented for several marine crustaceans (e.g., Sandoz & Rogers, 1944; Costlow *et al.*, 1960, 1962, 1966; Knowlton, 1965; Ong & Costlow, 1970; Choudhury, 1971; Sandifer, 1973; Young & Hazlett, 1978; McKenney & Neff, 1979), little information is available concerning the individual and/or combined effects of these variables on larval metabolism. Most studies of larval respiration have dealt with non-palaemonid species (e.g., Vernberg & Costlow, 1966; Vernberg & Vernberg, 1970; Kutty *et al.*, 1971; DeCoursey & Vernberg, 1972; Belman & Childress, 1973; Sastry & McCarthy, 1973; Schatzlein & Costlow, 1978). Such studies of palaemonid larvae, and particularly of *Macrobrachium* larvae, are rare. Nelson *et al.* (1971) have studied temperature and salinity effects on the metabolic rate of juvenile *M. rosenbergii*, a developmental stage which customarily inhabits fresh water (Raman, 1967).

In the current study an attempt has been made to expand present knowledge of metabolic responses of palaemonid larvae to environmental variation by examining the influence of various combinations of salinity and temperature on the respiratory metabolism of the first zoeal stage of *M. holthuisi*, a larva restricted to brackish waters.

## Material and Methods

Ovigerous females of the freshwater palaemonid shrimp *M. holthuisi*, were maintained in aerated, river-water-filled aquaria in the laboratory. Specimens were collected from the Guaecá River (≈23°49′18″ S; 45°27′18″ W) which discharges into the São Sebastião Channel about 10 km south of São Sebastião in the state of São Paulo, Brazil. Larvae, usually released in the early evening, were collected and kept in small glass jars of river water (*S*, 0‰ at 20–25 °C; 200 larvae/l) for up to 3 days (moulting to the second zoeal stage did not occur during this period).

Cartesian diver microrespirometers (Holter, 1943), each having a total volume of between 8–13 μl, were used to measure the oxygen uptake of individual intermoult stage I zoeae in 24 different salinity and temperature combinations (0, 7, 14, 21, 28 and 35‰ salinity at 15, 20, 25 and 30 °C). Dilutions were prepared from filtered São Sebastião Channel sea water and Guaecá River water. Oxygen uptake was measured each 30 min over a 2.5-h period, the first reading for each diver was disregarded. For each combination, a minimum of seven repetitions was performed, each zoea being used only once. Results are expressed as μl of oxygen consumed per mg dry weight per hour.

To determine the larval dry weight (calculated as 11.05 μg), three samples, each of 200 larvae were rinsed with distilled water, dried overnight at 80 °C, placed in a desiccator for 2 h and weighed using a Sartorius Model 2402 balance (0.01 mg sensitivity).

The data (with the exception of those combinations including 30 °C) were analysed statistically on an IBM 370 computer, at the University of South Carolina, using a

two-way analysis of variance program to determine whether respiration was significantly influenced by either salinity, temperature or the combined interaction of these two factors.

## RESULTS

Fig. 1 shows the effect of salinity on the rate of larval oxygen consumption at various temperatures. At 15 °C, the metabolic rates showed little variation with experimental salinity although a slight elevation occurred in river water (0‰ salinity). At 20 °C, a marked elevation occurred in the rate of oxygen consumption in river- and

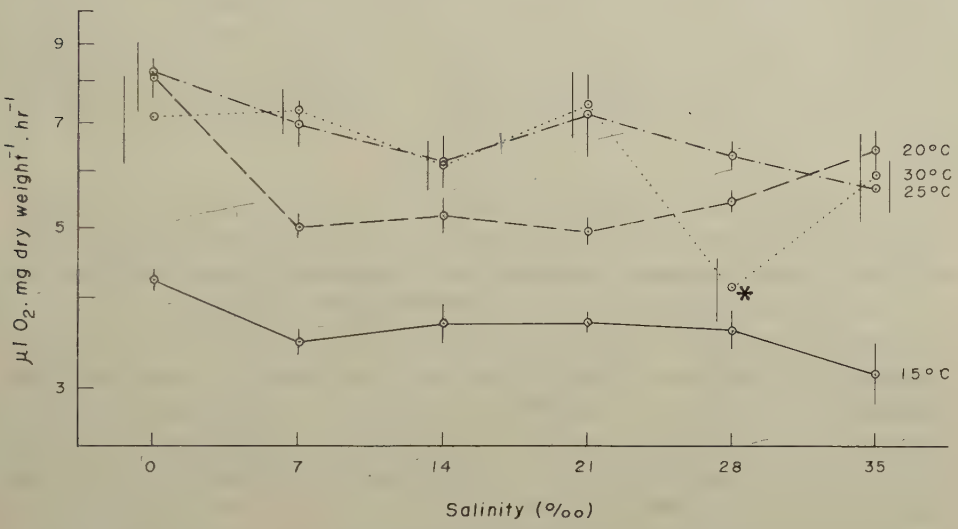


Fig. 1. Effect of salinity on the metabolic rates of stage I *Macrobrachium holthuisi* zoeae, in different temperatures. (* 21 replicates were performed for this combination.)

undiluted sea waters (*S*, 0 and 35‰); little variation was recorded in the intermediate salinities, resulting in a broad U-shaped curve, with the river-water end of the curve showing the greatest elevation. A slight elevation in metabolic rate occurred in river water at 25 °C. The rates remained constant, however, in the other salinities at this temperature. At 30 °C, metabolic rates were approximately constant over the experimental salinities used although an incongruous drop occurred at 28‰. In contrast to the elevation in metabolic rates recorded in river water at 15, 20 and 25 °C, a slight depression was noted at 30 °C.

Temperature has a readily observable effect on larval respiratory metabolism. At 15 °C, the rate of oxygen consumption ranged from 3.15 to 4.25 $\mu$l $O_2 \cdot$ mg dry $wt^{-1} \cdot h^{-1}$ and at 20 and 25 °C maximum $Q_{O_2}$ values of 8.16 and 8.20 $\mu$l $O_2 \cdot$ mg dry

wt $^{-1} \cdot h^{-1}$ were obtained respectively. At 30 °C, the $Q_{O_2}$ values varied from 4.14 to 7.49 $\mu$l $O_2 \cdot$ mg dry $wt^{-1} \cdot h^{-1}$. A decrease was noted in comparison with the values recorded at 25 °C in the extreme salinities, indicating that the combination of high temperature and low or high salinities has a deleterious effect on larval metabolic activity (Table I).

TABLE I

Metabolic rates ($\mu$l $O_2 \cdot$ mg dry weight$^{-1} \cdot h^{-1}$) of stage I *Macrobrachium holthuisi* zoeae in different temperature and salinity combinations.

| Temperature (°C) | Salinity (‰) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 35 |
| 15 | 4.25 ± 0.15 | 3.49 ± 0.13 | 3.70 ± 0.24 | 3.71 ± 0.10 | 3.62 ± 0.27 | 3.15 ± 0.33 |
| 20 | 8.16 ± 0.51 | 5.08 ± 0.19 | 5.23 ± 0.35 | 4.99 ± 0.24 | 5.44 ± 0.19 | 6.49 ± 0.40 |
| 25 | 8.20 ± 0.94 | 7.00 ± 0.56 | 6.22 ± 0.21 | 7.27 ± 1.07 | 6.35 ± 0.29 | 5.71 ± 0.53 |
| 30 | 7.15 ± 1.07 | 7.30 ± 0.54 | 6.21 ± 0.49 | 7.49 ± 0.80 | 4.14 ± 0.46 | 5.93 ± 0.88 |

TABLE II

$Q_{10}$ values for the respiratory rates of stage I *Macrobrachium holthuisi* zoeae in different salinities.

| Salinity (‰) | Temperature range (°C) | | |
|---|---|---|---|
| | 15–20 | 20–25 | 25–30 |
| 0 | 3.69 | 1.01 | – |
| 7 | 2.12 | 1.90 | 1.09 |
| 14 | 2.00 | 1.41 | 1.00 |
| 21 | 1.81 | 2.12 | 1.06 |
| 28 | 2.26 | 1.36 | – |
| 35 | 4.24 | – | – |

TABLE III

Analysis of variance demonstrating the effects of temperature and salinity on the metabolic rates of stage I *Macrobrachium holthuisi* zoeae.

| Variable* | d.f. | Sum of squares | Mean square | *F* | *P* value |
|---|---|---|---|---|---|
| Temperature | 2 | 7.685 | 3.840 | 95.05 | <0.01 |
| Salinity | 5 | 1.145 | 0.229 | 5.66 | <0.01 |
| Interaction (T × S) | 10 | 0.926 | 0.0926 | 2.29 | <0.02 |
| Error | 105 | 4.245 | 0.0404 | | |
| Total | 122 | 14.00 | | | |

* The dependent variable was the logarithm of the weight-specific oxygen consumption.

$Q_{10}$ values (Table II) decreased with increasing temperature, the highest values being observed in the temperature range 15–20 °C in all salinities with the exception of 21‰, where a slightly higher value was observed over the 20–25 °C range. In general, $Q_{10}$ values were higher in the extreme salinities (0 and 35‰) over the 15–20 °C range. Over the 20–25 °C range, however, $Q_{10}$ values showed no obvious trend and were moderately constant. $Q_{10}$ values could not be calculated for the extreme salinities (0, 28 and 35‰) in the 25–30 °C temperature range due to the decrease in metabolic rates in these experimental combinations.

The results of the analysis of variance are shown in Table III. The F-values for the variation in $Q_{O_2}$ due to temperature and salinity indicated a significant effect due to each of these components at the 0.01 level and for the interaction component at the 0.02 level, although that of temperature predominated. The model explained 69.7% of the total variation in the data.

## Discussion

The effects of temperature and salinity on the metabolic responses of marine invertebrates have been thoroughly discussed by many authors (see Kinne, 1970, 1971 and Vernberg & Vernberg, 1972 for reviews) while both Florkin (1960) and Lockwood (1967) have given special attention to these effects in crustaceans.

Statistical analyses have shown that although salinity, temperature, and their interaction significantly influence the metabolic rate of the first zoeal stage of *M. holthuisi*, the effect of temperature is much more pronounced. *M. holthuisi* is a subtropical species and both adults and larvae probably only occasionally encounter temperatures below 20 °C in their natural habitats. The annual variations recorded for the Guaecá River (1975–1976) and the São Sebastião Channel (1971–1972) were 17.5–28.5 °C (Lobão, 1977) and 18.5–27.5 °C (Yamashita, 1977), respectively. As might be expected then, larval $Q_{10}$ values were usually higher at temperatures below that of the normal habitat range as has been described for other marine crustaceans (Teal, 1959; Vernberg, 1959; Winget, 1969; Moreira, 1976; Moreira & Vernberg, 1978).

Despite the fact that the metabolism of *M. holthuisi* zoeae shows a marked salinity independence over the 7–28‰ range at 20 °C, elevated metabolic rates were recorded in fresh and sea waters (0 and 35‰). Significantly, laboratory studies have shown that the first and second zoeae of this species survive poorly in these salinities, although adults customarily inhabit fresh water (Moreira *et al.*, 1979). Like many other *Macrobrachium* larvae, the early zoeae of *M. holthuisi* also require brackish water for complete development. Laboratory studies also have shown that highest survival occurs at about 20 °C, the temperature most frequently encountered in the larval habitat (Moreira *et al.*, 1979). We consider, therefore, that the most typical effect of salinity would be that expressed at 20 °C. The high $Q_{O_2}$ values in the

extreme salinities at this temperature may be partly explained by an increased energy expenditure for osmoregulation which might permit short term larval survival in river water (where hatching may occur) or in coastal sea water to which the larvae may be carried by currents.

Kinne (1971) discussed several types of metabolic response to salinity exhibited by marine and brackish water invertebrates. According to his scheme, the response of *M. holthuisi* zoea I to salinity variation corresponds to "type (ii)" in which metabolism increases in sub- and supra-normal salinities. Many other crustaceans exhibit this particular response, among them *Ocypode albicans* (Flemister & Flemister, 1951), *Palaemonetes varians* (Lofts, 1956), *Metapanaeus monoceros* (Rao, 1958), and *Penaeus indicus* (Kutty *et al.*, 1971). In contrast, Moreira & Vernberg (1978) reported that the planktonic marine copepod *Euterpina acutifrons* from the São Sebastião Channel shows a decrease in $Q_{O_2}$ values in salinities greater and lower than 35‰ (normal) over the temperature range 20–32 °C (type (iii)- Kinne, 1971). This response is perhaps attributable to the fact that this species is not exposed to as wide a range of salinity variation as is *Macrobrachium holthuisi* during its life cycle.

The metabolic rate of *M. holthuisi* zoeae did not significantly vary over the salinity range used at 15 °C. However, although larvae appear to regulate their metabolism at this temperature, they also show very poor survival and are unable to moult to the second zoeal stage (Moreira *et al.*, 1979). Larval metabolism appears to be salinity independent over the salinity range normally encountered in the larval biotope (7–21‰) at the experimental temperatures used (15–30 °C).

The results obtained by Nelson *et al.* (1977) studying *M. rosenbergii* juveniles reared at 24 ± 1 °C, differ markedly from those recorded in this study for *M. holthuisi* larvae when the metabolic responses of both organisms are compared at 20 °C over a similar salinity range. Metabolic rates of *M. rosenbergii* juveniles are approximately constant between salinities of from 0–28‰ while those of *M. holthuisi* larvae, as previously noted, take the form of a broad U-shaped curve over the 0–35‰ salinity range. Considering the general migration to fresh water from brackish water occurring shortly after metamorphosis in both *M. holthuisi* (McNamara & Moreira, unpublished data) and *M. rosenbergii* (Ling, 1969), it is most likely that differences in larval and juvenile metabolic responses to salinity variations would occur.

Clearly, further studies of larval osmoregulatory capability (e.g., Sandifer *et al.*, 1975) and the effects of salinity on the survival and metabolism of the developmental stages of *Macrobrachium* species are necessary to elucidate the complex physiological modifications which initiate the migration of larvae and juveniles to their different biotopes.

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## References

Beiman, B. W. & J. J. Childress. 1973. Oxygen consumption of the larvae of the lobster *Panulirus interruptus* (Randall) and the crab *Cancer productus* Randall. *Comp. Biochem. Physiol.*, Vol. 44A, pp. 821–828.

Choudhury, P. C., 1970. Complete larval development of the palaemonid shrimp *Macrobrachium acanthurus* (Wiegmann, 1836), reared in the laboratory. *Crustaceana*, Vol. 18, pp. 113–132.

Choudhury, P. C., 1971. Responses of larval *Macrobrachium carcinus* (L.) to variations in salinity and diet (Decapoda, Palaemonidae). *Crustaceana*, Vol. 20, pp. 113–120.

Costlow, J. D. Jr, C. G. Bookhout & R. Monroe, 1960. The effect of salinity and temperature on the larval development of *Sesarma cinereum* (Bosc) reared in the laboratory. *Biol. Bull. mar. biol. Lab., Woods Hole*, Vol. 118, pp. 183–202.

Costlow, J. D. Jr, C. G. Bookhout & R. Monroe, 1962. Salinity temperature effects on the larval development of the crab. *Panopeus herbstii* Milne-Edwards, reared in the laboratory. *Physiol. Zoöl.*, Vol. 35, pp. 79–93.

Costlow, J. D. Jr, C. G. Bookhout & R. Monroe, 1966. Studies on the larval development of the crab. *Rhithropanopeus harrisii* (Gould). I. The effect of salinity and temperature on larval development. *Physiol. Zoöl.*, Vol. 39, pp. 81–100.

DeCoursey, P. J. & W. B. Vernberg, 1972. Effect of mercury on survival, metabolism and behavior of larval *Uca pugilator*. *Oikos*, Vol. 23, pp. 241–247.

Flemister, L. J. & S. C. Flemister, 1951. Chloride ion regulation and oxygen consumption in the crab *Ocypode albicans* (Bosc). *Biol. Bull. mar. biol. Lab., Woods Hole*, Vol. 101, pp. 259–273.

Florkin, M., 1960. Ecology and metabolism. In, *The physiology of Crustacea*, edited by T. H. Waterman, Academic Press, London, Vol. 1, pp. 395–410.

Holter, H., 1943. Technique of the Cartesian diver. *C.r. Lab. Carlsberg, Sér. Chim.*, Vol. 24, pp. 399–478.

Kinne, O., 1970. Temperature: animals: invertebrates. In, *Marine ecology, Vol. 1, Part 1*, edited by O. Kinne, Wiley-Interscience, London, pp. 407–514.

Kinne, O., 1971. Salinity: animals: invertebrates. In, *Marine ecology, Vol. 1, Part 2*, edited by O. Kinne, Wiley-Interscience, London, pp. 821–955.

Knowlton, R. E., 1965. Effects of some environmental factors on the larval development of *Palaemonetes vulgaris* (Say). *J. Elisha Mitchell sci. Soc.*, Vol. 81, p. 87.

Kutty, M. N., G. Murugapoopathy & T. S. Krishnan, 1971. Influence of salinity and temperature on the oxygen consumption in young juveniles of the Indian prawn *Penaeus indicus*. *Mar. Biol.*, Vol. 11, pp. 125–131.

Ling, S. W., 1969. The general biology and development of *Macrobrachium rosenbergii* (de Man). *FAO Fish. Rep.*, Vol. 57, pp. 589–606.

Lobão, V. L., 1977. Fisioecologia do Pitu, camarão d'agua doce – *Macrobrachium holthuisi* Genofre & Lobão, 1976. Crescimento, ciclo sexual, ciclo de muda em relação a fatores abióticos. Ph.D. thesis, Universidade de São Paulo, Brasil, 97 pp.

Lockwood, A. P. M., 1968. *Aspects of the physiology of Crustacea*. Oliver & Boyd, Edinburgh and London, 328 pp.

Lofts, B., 1956. The effects of salinity changes on the respiratory rate of the prawn *Palaemonetes varians* (Leach). *J. exp. Biol.*, Vol. 33, pp. 730–736.

McKenney, C. G. Jr & J. M. Neff, 1979. Individual effects and interactions of salinity, temperature, and zinc on the larval development of the grass shrimp *Palaemonetes pugio*. Survival and developmental duration through metamorphosis. *Mar. Biol.*, Vol. 52, pp. 177–188.

MONACO, G., 1975. Laboratory rearing of larvae of the palaemonid shrimp *Macrobrachium americanum* (Bate). *Aquaculture*, Vol. 6, pp. 369–375.

MOREIRA, G. S., 1976. Efeitos sinérgicos da temperatura e salinidade no metabolismo respiratório do copépode *Euterpina acutifrons* (Dana, 1847), da Baía de Santos e do Canal de São Sebastião. Livre-Docente thesis, Universidade de São Paulo, Brasil, 89 pp.

MOREIRA, G. S., J. C. MCNAMARA & P. S. MOREIRA, 1979. The combined effects of temperature and salinity on the survival and moulting of early zoeae of *Macrobrachium holthuisi* (Decapoda: Palaemonidae). *Bol. Fisiol. Animal, São Paulo*, Vol. 3, pp. 81–93.

MOREIRA, G. S. & W. B. VERNBERG, 1978. Synergistic effects of environmental variables on the metabolism of the copepod *Euterpina acutifrons* from two different areas off the coast of the State of São Paulo, Brazil. *Fish. Bull. nat. mar. Fish. Service*, Vol. 76, pp. 449–455.

NELSON, S. G., D. A. ARMSTRONG, A. W. KNIGHT & H. W. LI, 1977. The effects of temperature and salinity on the metabolic rate of juvenile *Macrobrachium rosenbergii* (Crustacea: Palaemonidae). *Comp. Biochem. Physiol.*, Vol. 56A, pp. 533–537.

ONG, K. S. & J. D. COSTLOW, JR, 1970. The effect of salinity and temperature on the larval development of the stone crab, *Menippe mercenaria* (Say), reared in the laboratory. *Chesapeake Sci.*, Vol. 11, pp. 16–29.

RAMAN, K., 1967. Observations on the fishery and biology of the giant freshwater prawn *Macrobrachium rosenbergii* de Man. *Proc. Symp. Crustacea, Mar. Biol. Ass., India, Ser. 2, Part II*, pp. 649–669.

RAO, K. P., 1958. Oxygen consumption as a function of size and salinity in *Metapenaeus monoceros* Fab. from marine and brackish water environments. *J. exp. Biol.*, Vol. 35, pp. 307–313.

SANDIFER, P. A., 1973. Effects of temperature and salinity on larval development of grass shrimp, *Palaemonetes vulgaris* (Decapoda, Caridea). *Fish. Bull. nat. mar. Fish. Service*, Vol. 71, pp. 115–123.

SANDIFER, P. A., J. S. HOPKINS & T. I. SMITH, 1975. Observations on salinity tolerance and osmoregulation in laboratory-reared *Macrobrachium rosenbergii* post-larvae (Crustacea: Caridea). *Aquaculture*, Vol. 6, pp. 103–114.

SANDOZ, M. & R. ROGERS, 1944. The effect of environmental factors on hatching, moulting, and survival of zoeae larvae of the blue crab, *Callinectes sapidus* Rathbun. *Ecology*, Vol. 25, pp. 216–228.

SASTRY, A. N. & J. F. MCCARTHY, 1973. Diversity in metabolic adaptation of pelagic larval stages of two sympatric species of brachyuran crabs. *Neth. J. Sea Res.*, Vol. 7, pp. 434–446.

SCHATZLEIN, F. C. & J. D. COSTLOW JR, 1978. Oxygen consumption of the larvae of the decapod crustaceans, *Emerita talpoida* (Say) and *Libinia emarginata* Leach. *Comp. Biochem Physiol.*, Vol. 61A, pp. 441–450.

TEAL, J. M., 1959. Respiration of crabs in Georgia salt marshes and its relation to their ecology. *Physiol. Zoöl.*, Vol. 32, pp. 1–14.

VERNBERG, F. J., 1959. Studies on the physiological variation between tropical and temperate zone fiddler crabs of the genus *Uca*. II. Oxygen consumption of whole organisms. *Biol. Bull. mar. biol. Lab., Woods Hole*, Vol. 117, pp. 163–184.

VERNBERG, F. J. & J. D. COSTLOW JR, 1966. Studies on the physiological variation between tropical and temperate-zone fiddler crabs of the genus *Uca*. IV. Oxygen consumption of larvae and young crabs reared in the laboratory. *Physiol. Zoöl.*, Vol. 39, pp. 36–52.

VERNBERG, W. B. & F. J. VERNBERG, 1972. *Environmental physiology of marine animals*. Springer-Verlag, New York, 346 pp.

WINGET, R. R., 1969. Oxygen consumption and respiratory energetics in the spiny lobsters *Panulirus interruptus* (Randall). *Biol. Bull. mar. biol. Lab., Woods Hole*, Vol. 136, pp. 301–312.

YAMASHITA, C., 1977. Flutuação populacional e tolerância a temperatura e salinidade do copépode *Euterpina acutifrons* (Dana, 1847), da região de Santos e do Canal de São Sebastião. Ph.D. thesis, Universidade de São Paulo, Brasil, 135 pp.

YOUNG, A. M. & T. L. HAZLETT, III, 1978. The effect of salinity and temperature on the larval development of *Clibanarius vittatus* (Bosc) (Crustacea : Decapoda : Diogenidae). *J. exp. mar. Biol. Ecol.*, Vol. 34, pp. 131–141.

*J. exp. mar. Biol. Ecol.*, 1980, Vol. 47, pp. 149–171
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# SELECTIVE SITE SEGREGATION IN *PATELLOIDA (CHIAZACMEA) PYGMAEA* (Dunker) AND *P.(C.) LAMPANICOLA* Habe (GASTROPODA: PATELLACEA) ON A HONG KONG SHORE

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**Abstract:** Although originally described as a separate species, *Patelloida (Chiazacmea) lampanicola* Habe has subsequently been regarded as a "form" of *P. (C.) pygmaea* (Dunker). The conical shape of the shell in the former was assumed to result from the settlement and growth of some individuals of the population upon shells of intertidal *Batillaria* (Potamididae).

This paper reports upon an analysis of a population of limpets resident on a small sand flat in Hong Kong and it is concluded that *Patelloida (C.) lampanicola* is distinct from *P. (C.) pygmaea*. This conclusion is based upon differences in radula teeth structure, shell morphometrics and behaviour. Furthermore it has been demonstrated that the earlier confusion surrounding these species results from a very nice example of selective site segregation. *P. (C.) pygmaea* inhabits stones and empty shells (in this region of its total range) embedded in the sand but can and occasionally does reside, up to a length of 4.2 mm, upon *Batillaria* shells. Thereafter the flattened form of the limpet shell, on a round substratum, presumably makes such individuals more susceptible to either predation or dislodgement. The high-coned *Patelloida (C.) lampanicola*, on the other hand, almost exclusively colonizes living *Batillaria* (particularly *B. zonalis* and *B. multiformis*) shells but can, with little or no significant alteration in form, also colonize stones. *Patelloida (C.) lampanicola* has a positive behavioural response towards *Batillaria* and is clearly living in symbiotic association with the potamid. The benefits accrued by both are discussed.

Thus, the limited hard substratum of a primarily soft shore environment has been segregated by these two limpets; one occupies the stones and oyster shells, the other the dense cover of epifaunal potamid snails.

## INTRODUCTION

The acmaeid limpet *Patelloida (Chiazacmea) pygmaea* (Dunker, 1861) (Fig. 1A) is widely distributed throughout the Indo-Pacific and in Hong Kong is found on sheltered shores (with a distinct estuarine influence) attached to rocks, empty rock oysters (*Saccostrea cucullata* Born) and, on predominately soft shores, to stones and empty shells (often similarly covered in oyster shells). The shell of *Patelloida pygmaea* is variable and a number of species are possibly either synonymous or have been confused with it (Habe, 1944).

Habe (1944) originally described *P. lampanicola* (Fig. 1B) as a distinct species but later (Habe, 1971) considered it to be but a "form" of *P. pygmaea*, obtaining its distinctive conical shape by residing upon the shells of species of *Batillaria;* its flattened counterpart (i.e. *Patelloida pygmaea*) residing on stones. A preliminary series of observations on *P. pygmaea* and *P. lampanicola* in their natural environment

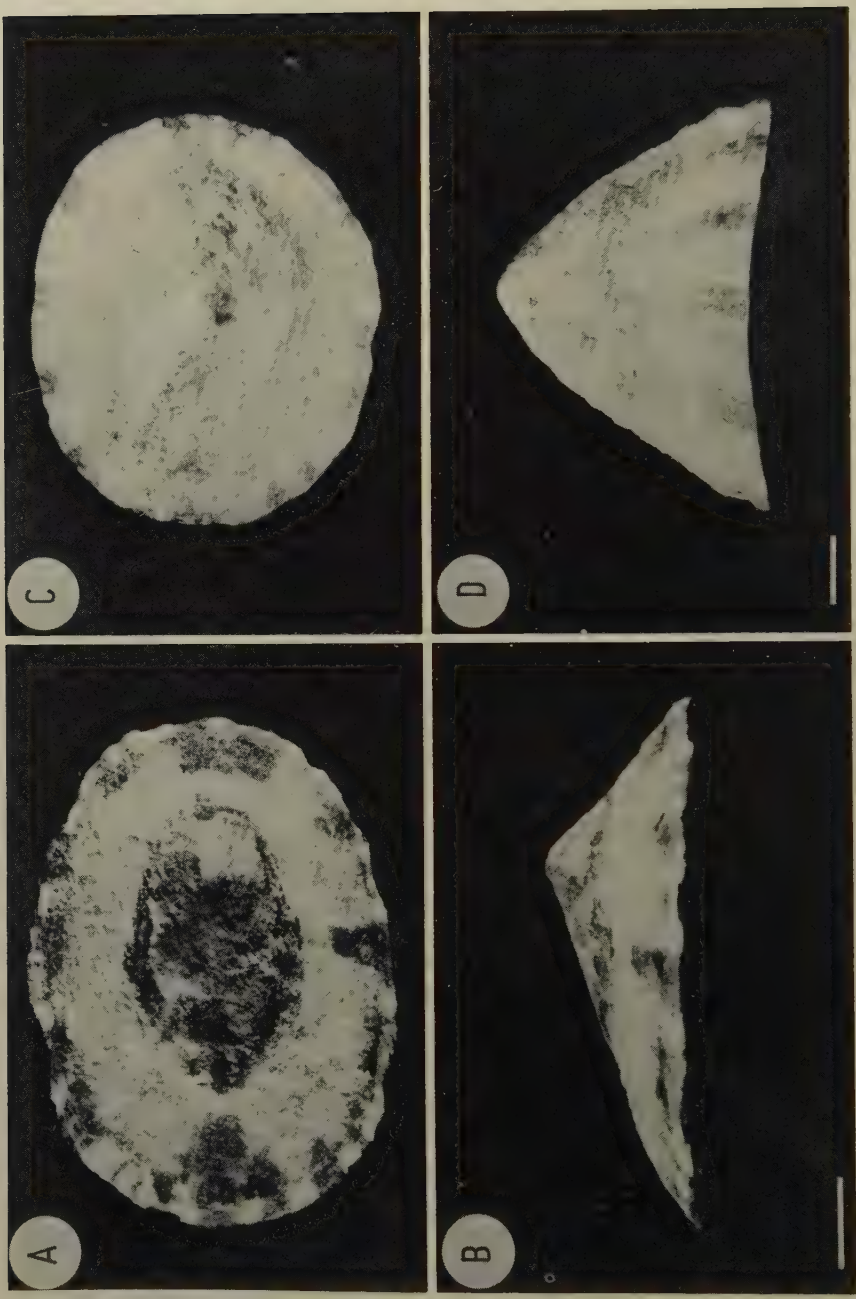


Fig. 1. Dorsal and lateral views of *Patelloida pygmaea* (A and B) and *P. lampanicola* (C and D) scale bar 1 mm.

indicated that the latter interpretation was untrue because flattened *P. pygmaea* could occasionally be found on *Batillaria* and conical *Patelloida lampanicola* on stones. Accordingly, a study of a population of these two limpets has been undertaken at Tai Tam Bay, Hong Kong, with the object of testing whether or not *P. pygmaea* does exist as two growth forms, mediated by the choice of substratum or if there are two species, dividing a niche. In either case an understanding and definition of the nature of the relationship between *P. lampanicola* and *Batillaria* is required.

## FIELD OBSERVATIONS

Every month for a period of 13 months commencing in January 1977, 0.25-$m^2$ samples of *Batillaria* were collected at 1-m intervals down a 30-m transect (A–A′) (Fig. 2) on a sand bar in Tai Tam Bay, Hong Kong Island. Three species were present: *B. zonalis* (Bruguière), *B. multiformis* (Lischke), and *B. cumingii* (Crosse). The samples were divided into species and for December 1977 (when the snails were in the closing stages of a phase of burial and likely to be occurring in a typical pattern on the shore) the pattern of distribution and population structure of the component species were assessed. Each specimen of each species was measured along its greatest length and inspected for limpets. The latter were identified as either *Patelloida lampanicola* or *P. pygmaea* and their position on each specimen of each species of *Batillaria* accurately recorded on master diagrams (Fig. 6). Initially this was restricted to those limpets collected from the 0.25-$m^2$ quadrats, from where a total of 54 specimens of *Patelloida lampanicola* were recovered from 39,142 *Batillaria*. These data were augmented, however, by other collections from transect A–A′ undertaken during a further, wider survey of the Tai Tam sand flats to be described later. Specimens of *Patelloida lampanicola* were measured to the nearest 0.5 mm using dial calipers. These data have been correlated with aspects of the behaviour of *Batillaria* as recorded in field notes and with climatological and hydrological data for 1977.

During the summer of 1977, a further series of transects was laid out across the sand bars in Tai Tam Bay (Fig. 2). At one metre intervals along each transect, a 0.25-$m^2$ quadrat was laid down and a number of records taken: (1) whether the substratum was mud, sand with $<10\%$ or sand with $>10\%$ cover of stones or empty shells (Fig. 2A); (2) the number of *Batillaria*/0.25-$m^2$ (Fig. 2B); (3) the numbers of *Patelloida pygmaea*/0.25-$m^2$ (either on stones and empty shells or on *Batillaria*) (Fig. 2C); and (4) the numbers of *Patelloida lampanicola*/0.25-$m^2$ (either on stones and empty shells or on *Batillaria*) (Fig. 2D). From these data a series of distribution maps of substratum type, and of the two limpets has been built up.

A
B
N
10 m
Mud
Water
Vegetation on high ground
Stones > 10% cover
Stones < 10% cover
Water
Batillaria >600/m²
Batillaria >100, <600/m²
Batillaria <100/m²

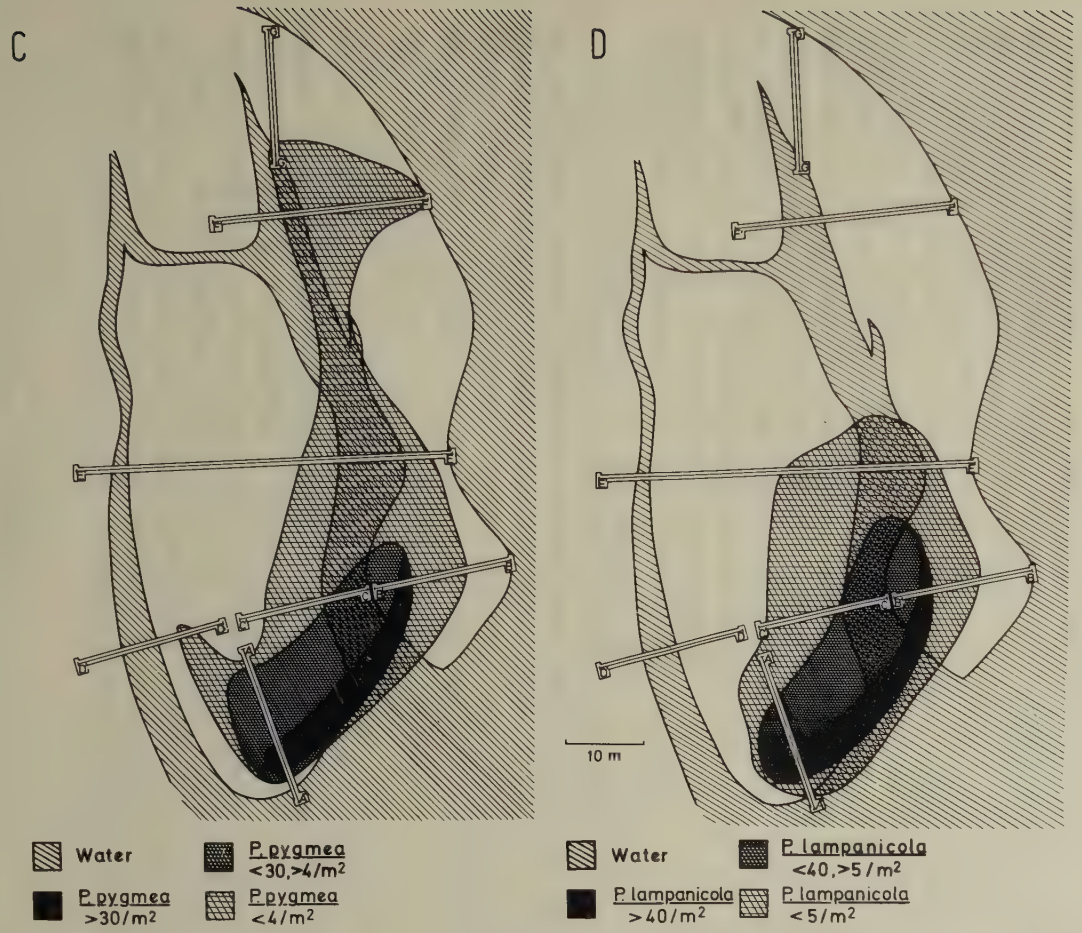


Fig. 2. Diagrams of the sand bars in Tai Tam Bay, Hong Kong Island, showing the distribution of different deposits and vegetation (A), potamid snails (B), *Patelloida pygmaea* (C), and *P. lampanicola* (D): the transects are also shown.

### LABORATORY EXPERIMENTS

In the laboratory, 20 individuals of each of the two limpets were released into an inverted Petri dish in a tank of sea water and allowed to acclimate. Around the dish were arranged, in each quarter, either living *Batillaria*, stones, gravel or empty bivalve shells. After 1 h, the Petri dish was removed, so that the limpets were free to explore the surrounding different quadrats. Their positions after 2 h and after 2 days was recorded. The numbers of *Patelloida lampanicola* and *P. pygmaea* on either *Batillaria* or on all other grouped substrata was tested using the $2 \times 2$ test of independence ($G$ test with the Yate's correction) (Sokal & Rohlf, 1969).

As a second experiment 20 limpets of each species (or form) were invited, using the same choice experiment, to choose between quadrats containing gravel or twenty, similar sized, specimens of either *B. zonalis*, *B. multiformis* or *B. cumingii*. This experiment was repeated. The data obtained from both experiments were pooled and for each species the results were tested for goodness of fit using the $\chi^2$ test. This determined whether each species demonstrated a substratum preference.

The data obtained for both species were then compared, to see if their choice of substratum was different, using the $R \times C$ test of independence ($G$ test) (Sokal & Rohlf, 1969).

As a third experiment an inverted Petri dish, containing 20 individuals each of both limpets was surrounded solely by stones embedded in gravel. The distribution of the limpets was measured after 2 h and 2 days. The numbers of *Patelloida lampanicola* and *P. pygmaea* on either the stones and the dish or the gravel and the tank after 2 days was tested using the $2 \times 2$ test of independence ($G$ test with the Yate's correction) (Sokal & Rohlf, 1969).

### RADULA STRUCTURE

Specimens, separated by differences in shell form and assignable with certainty to either *P. pygmaea* or *P. lampanicola*, were dissected and the radula removed. Surrounding tissue was removed using household bleach and temporary preparations made. Camera lucida diagrams were made of the teeth from the anterior, middle, and posterior regions and were compared. Once criteria for separating them had been recognized, studies of the radula proved the ultimate diagnosis for small, deformed or eroded individuals.

## RESULTS

### TAXONOMY

*P. pygmaea* was originally described as *Patella pygmaea* Dunker 1861. It was formerly thought to be synonymous with *Patella heroldi* (Dunker, 1861) by Lischke (1869, 1871) and Pilsbry (1891, 1895); the latter species is now, however, placed in

:nus *Collisella* (Habe, 1971). Habe (1944) also placed *Patelloida pygmaea* in subgenus *Asteracmaea* (erected by Oliver, 1926), but later changed his mind (Habe, 1971) and placed it in *Chiazacmea* (also erected by Oliver, 1926). One of the species under consideration is thus, at present, *Patelloida (Chiazacmea) pygmaea* (Dunker 1861) (Fig. 1A, B).

Similarly, *P. (Chiazacmaea) lampanicola* Habe 1944 (Fig. 1C, D), was originally assigned to the subgenus *Asteracmaea* (Habe, 1971). The species is similar to *Patelloida conulus* (Dunker, 1861) (Habe, 1944) which in turn has also been synonymized with *P. heroldi* (Dunker, 1861), the latter taking nomenclatorial precedence. At one time or other, therefore, the *P. pygmaea*, *P. conulus* and *P. heroldi* of Dunker have all been synonymized which may have led Habe (1971) (and Kuroda *et al.*, 1971) to eventually conclude that his own species *Patelloida lampanicola* was but a "form" of *P. pygmaea*, the former occurring on *Batillaria* shells, the latter on stones. Similarly, Habe (1971) considered *P. signata* (Pilsbry 1901) to be a deep water "form" of *P. pygmaea*.

## THE SHELL

The shell of *P. pygmaea* (Fig. 1A, B) is in the form of an oval, low cone with the apex towards the anterior. Characteristically, four bands of pigment radiate from the apex, although the shell is polymorphic and the bands may be subdivided into a greater number. The brown rays characterize the subgenus *Chiazacmea* (Oliver, 1926). The apex of the shell is typically eroded and because of this the bands are never continuous from apex to margin. Internally the shell possesses a dark brown apex and scar, while the remainder of the shell is translucent white.

*Patelloida lampanicola* (Fig. 1C, D) is in the form of an oval to circular, tall cone with the apex shifted only very slightly to the anterior. The outer surface is lined with radiating brown pigment bands although presumably because of intense wear such pigment is only visible at the margin. The remainder of the shell is typically from translucent white to yellow. Internally the muscle scar is a pale tan.

The ratio of shell length to height has been effectively used for *Patella* by Moore (1934) and for *Cellana* by Rao & Ganapati (1970) to demonstrate disparity in growth between high- and low-zoned populations of these intertidal limpets. Giesel (1969) has used the same criteria to separate sub-populations of *Acmaea digitalis*.

This study, however, effectively demonstrates that specimens of *Patelloida lampanicola* on stones or *Batillaria* have approximately similar length: height ratios i.e. regression equations of respectively $y = x + 1.6$ and $y = 0.94x + 1.5$ which are virtually identical (Fig. 3). Clearly the tall *Patelloida lampanicola*, adapted to a normal mode of life on a rounded surface is not adversely limited by the irregular surface of a stone or oyster shell; its expression of growth on both substrata is thus very similar. On the other hand, *P. pygmaea* has a distinctive, flattened form

when occurring on stones. On *Batillaria*, however, the shell is somewhat taller in relation to length, presumably in an attempt to match in its own growth the effectively increasing roundness of the potamid shell. In this attempt to adapt to a different substratum *Patelloida pygmaea* is clearly unsuccessful because no specimens of this species > 4.2 mm in length have been found on *Batillaria*. Presumably the

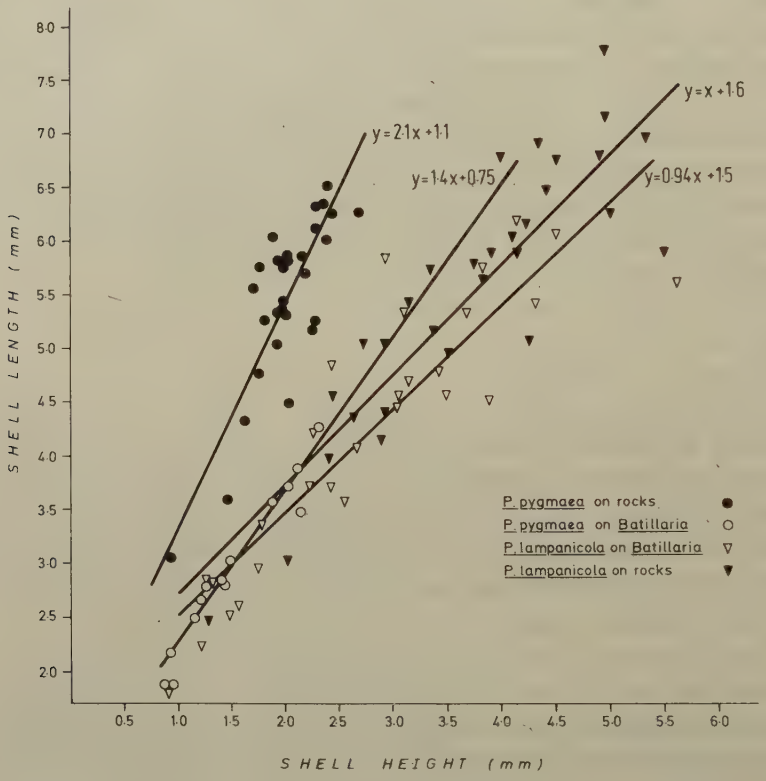


Fig. 3. Relation between shell length and height of *Patelloida pygmaea* on stones or shells, *P. pygmaea* on *Batillaria*, *P. lampanicola* on *Batillaria*, and *P. lampanicola* on stones.

inherent plasticity in the form of the shell of *Patelloida pygmaea* is insufficient to allow it to match the growth pattern of *P. lampanicola*. Eventually a gap must develop between the shell of *P. pygmaea* and the potamid, facilitating either dislodgement or predation. A mechanism for selection is clearly apparent here.

## THE RADULA

The radula of *Patelloida* comprises, on each side, a pair of very fine marginal teeth, a bicuspid exterior lateral tooth and a unicuspid interior lateral tooth. The teeth of *P. pygmaea* have been described by Habe (1944) and of other species of

*Patelloida* by Christiaens (1975a, b, 1976a, b). The radulae from many specimens of *P. pygmaea* and *P. lampanicola* have been examined in this study. Fig. 4 compares a row of teeth of *P. lampanicola* (Fig. 4A) and *P. pygmaea* (Fig. 4B) seen from above and the lateral teeth seen from the side. Both illustrations represent camera lucida drawings of the teeth from the central region of the radula. As in all limpets, proximal and distal teeth vary in size and micro-structure, but the teeth of the two species are consistently different. The drawing of the teeth of *P. pygmaea* (Fig. 4B) is very similar to that of *P. pygmaea* illustrated by Habe (1944, Fig. 2) and

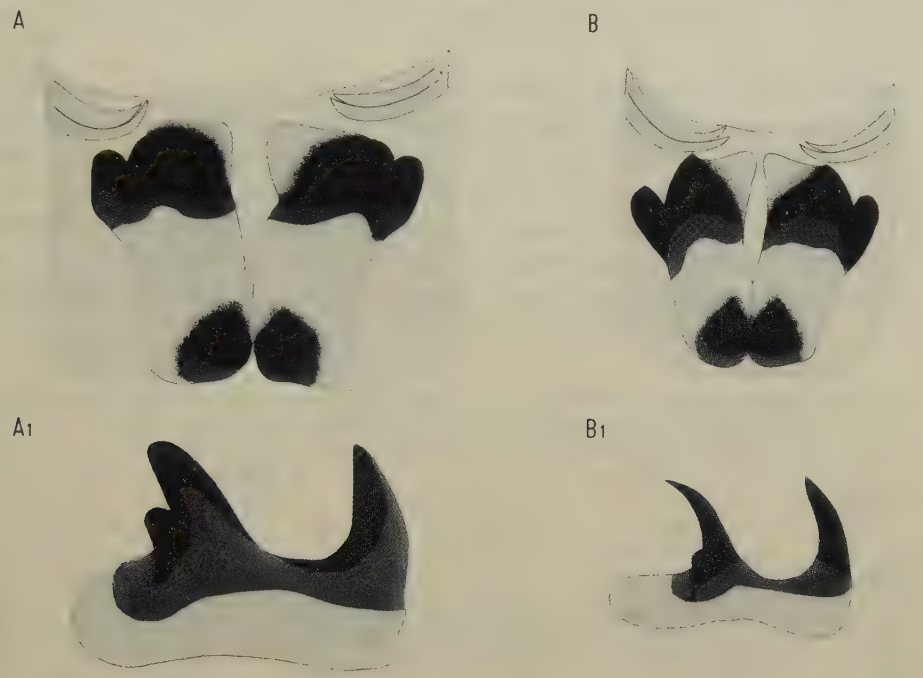


Fig. 4. Camera lucida drawings of the radula teeth as seen from above and the side: A, $A_1$, *P. lampanicola*; B, $B_1$, *P. pygmaea*.

demonstrates that the cusps of the external lateral teeth are sharply pointed (particularly the innermost). The teeth, as seen laterally (Fig. $4B_1$) are also very delicate. In contrast the lateral teeth of *P. lampanicola* are altogether much stouter (Fig. 4A, $A_1$) and the cusps of the external laterals are broad and spade like. Runham & Thornton (1967) have shown that in *Patella vulgata* unworn teeth have tall, pointed cusps whereas worn teeth are chisel-shaped. The differences in cusp shape, however, between *Patelloida pygmaea* and *P. lampanicola* (whether occupying stones or *Batillaria* shells) do not result from enhanced wear in the latter species (indeed, one would expect *Patelloida lampanicola*, occupying a possibly "softer" substratum, to have less worn teeth than *P. pygmaea* on stones), but represent a

genuine difference enabling clear separation of the species. Specimens tentatively ascribed to one species on the basis of shell characters could always be confirmed by examination of the radulae.

## FIELD STUDIES

On the sand flats of Tai Tam Bay, Hong Kong Island, the epifauna is dominated by three species of *Batillaria* i.e. *B. zonalis*, *B. multiformis*, and *B. cumingii*. These gastropods usually act as a substratum for *Patelloida lampanicola*. *P. pygmaea* typically attaches to stones or empty bivalve shells (*Saccostrea cucullata*) in or on the sand.

Fig. 2C, D correlates the distribution of *Patelloida pygmaea* and *P. lampanicola* with that of stones or empty shells and *Batillaria* (Fig. 2A, B) on two small sand bars in Tai Tam Bay. It can be seen that both *Patelloida pygmaea* and *P. lampanicola* occur in greatest numbers at the mouth of a small stream draining into the Bay; this possibly reflects a similar salinity optimum. *P. pygmaea*, however, in its wider distribution, matches more approximately the areas of stones and shells on the sand matrix. It does not, significantly, match the wider pattern of *Batillaria* distribution.

TABLE I

The numbers and percentages of limpets of both species upon either stones and shells or *Batillaria* as recorded from each transect in Tai Tam Bay during the summer of 1977.

| Transect | *P. pygmaea* | | *P. lampanicola* | | |
|---|---|---|---|---|---|
| | Numbers | % | Numbers | % | Total |
| Limpets on *Batillaria* | | | | | |
| A | 16 | 1.5 | 90 | 85.0 | 106 |
| F | 1 | 12.5 | 7 | 87.5 | 8 |
| E | 1 | 4.8 | 20 | 95.2 | 21 |
| G | – | – | – | – | – |
| H | – | – | 10 | 100 | 10 |
| B | – | – | – | – | – |
| D | – | – | – | – | – |
| Total | 18 | 12.4 | 127 | 87.6 | 145 |
| Limpets on stones and shells | | | | | |
| A | 10 | 83.3 | 2 | 16.7 | 12 |
| F | 57 | 53.3 | 50 | 46.7 | 107 |
| E | 13 | 72.2 | 5 | 27.8 | 18 |
| G | 1 | 100 | – | – | 1 |
| H | 3 | 75.0 | 1 | 25.0 | 4 |
| B | 4 | 100 | – | – | 4 |
| D | 1 | 100 | – | – | 1 |
| Total | 89 | 60.5 | 58 | 39.5 | 147 |

TABLE II

Raw data showing the incidence of *Patelloida lampanicola* upon the various species of *Batillaria* collected at monthly intervals from transect A–A′ in Tai Tam Bay.

| | | *P. lampanicola* | | | *P. lampanicola* | | | *P. lampanicola* | | Total | Total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *B. zonalis* | No. | % | *B. multiformis* | No. | % | *B. cumingii* | *No.* | *%* | *Batillaria* | *P. lampanicola* | % frequency |
| January | 1412 | 6 | 0.42 | 608 | 4 | 0.66 | 29 | – | – | 2049 | 10 | 0.48 |
| February | 1058 | 5 | 0.47 | 710 | 1 | 0.14 | 29 | – | – | 1797 | 6 | 0.33 |
| March | 1209 | 6 | 0.50 | 1013 | 2 | 0.20 | 25 | – | – | 2247 | 8 | 0.36 |
| April | 1381 | 1 | 0.07 | 1199 | – | – | 72 | – | – | 2652 | 1 | 0.04 |
| May | 1159 | 2 | 0.17 | 490 | 1 | 0.20 | 50 | – | – | 1699 | 3 | 0.18 |
| June | 1311 | 2 | 0.15 | 657 | – | – | 56 | – | – | 2024 | 2 | 0.10 |
| July | 854 | 4 | 0.47 | 1032 | 1 | 0.10 | 65 | – | – | 1951 | 5 | 0.26 |
| August | 1627 | 10 | 0.61 | 385 | – | – | 114 | 1 | 0.008 | 2126 | 11 | 0.52 |
| September | 1586 | – | – | 1656 | – | – | 156 | – | – | 3398 | – | 0 |
| October | 2227 | 1 | 0.04 | 485 | – | – | 127 | – | – | 2839 | 1 | 0.04 |
| November | 2687 | 1 | 0.04 | 1616 | – | – | 163 | – | – | 4466 | 1 | 0.02 |
| December | 3588 | 1 | 0.03 | 2497 | – | – | 268 | – | – | 6353 | 1 | 0.02 |
| January | 2293 | 5 | 0.22 | 3028 | – | – | 220 | – | – | 5541 | 5 | 0.09 |
| Total/mean | 22,392 | 44 | 0.24 | 15,376 | 9 | 0.10 | 1374 | 1 | 0.0006 | 39,142 | 54 | 0.19 |

*Patelloida lampanicola*, on the other hand, has a narrower overall distribution, does not extend onto the stony areas at the head of the inlet and matches the distribution of *Batillaria* particularly on the eastern component of the sand bars. It does not follow the major *Batillaria* population (mostly comprising *B. cumingii*) onto the western arm of the sand bar. No specimens of either *Patelloida pygmaea* or

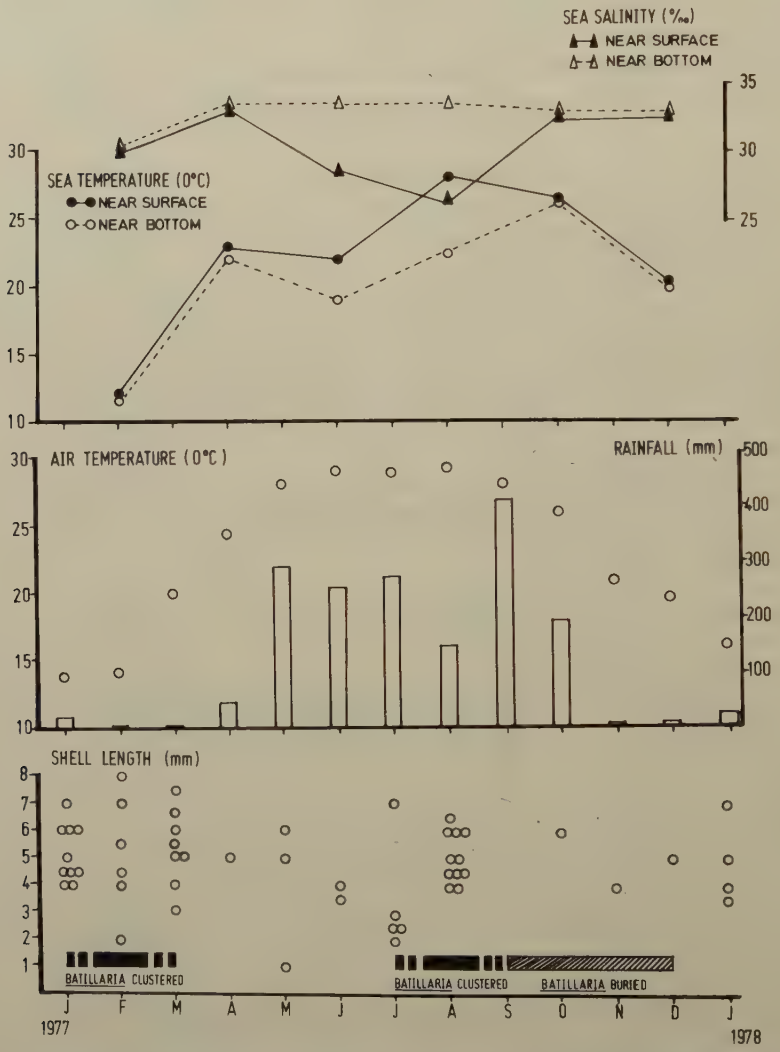


Fig. 5. The incidence of *P. lampanicola* collected from transect A–A′ every month between January 1977 and January 1978: each individual was measured along its greatest length; characteristic aspects of the behaviour of *Batillaria* are also recorded; this information is correlated with data on air temperature and rainfall for Hong Kong and with sea surface and bottom temperatures and salinities for Tai Tam Bay over the same period; climatological data provided by the Royal Observatory, Hong Kong, hydrological data by the Public Works Department of the Hong Kong Government.

*P. lampanicola* were found on stones or on *Cerithidea rhizophorarum* (A. Adams) that colonizes the area of mud to the west.

Table I shows the actual numbers and percentages of specimens of *Patelloida pygmaea* and *P. lampanicola* on either stones or *Batillaria* throughout the seven transects. It can be seen that a total of 145 limpets were recovered from *Batillaria* and 147 from the stones. Of the limpets recovered from *Batillaria*, 127 or 87.6% were *Patelloida lampanicola*. Of the limpets recovered from stones or empty shells 89 or 60.5% were *P. pygmaea*. These data effectively demonstrate the affinity of *P. lampanicola* for *Batillaria* but also demonstrate that the species can live on stones. Conversely *Patelloida pygmaea* is largely found on stones but can occur on potamid snails, although as demonstrated earlier, the individuals which do colonize *Batillaria* are always small.

Analysis of these data in a different manner demonstrates that over 83% of all the specimens of *Patelloida pygmaea* were recovered from stones whereas 68.6% of all specimens of *P. lampanicola* were recorded from *Batillaria*. Again *Patelloida lampanicola* was consistently found on stones (31.7%) more than *P. pygmaea* was found on *Batillaria* (16.8%).

Every month throughout 1977, 0.25-$m^2$ quadrat samples of *Batillaria* were collected from 1-m intervals down transect A–A′. An average of 3011 specimens

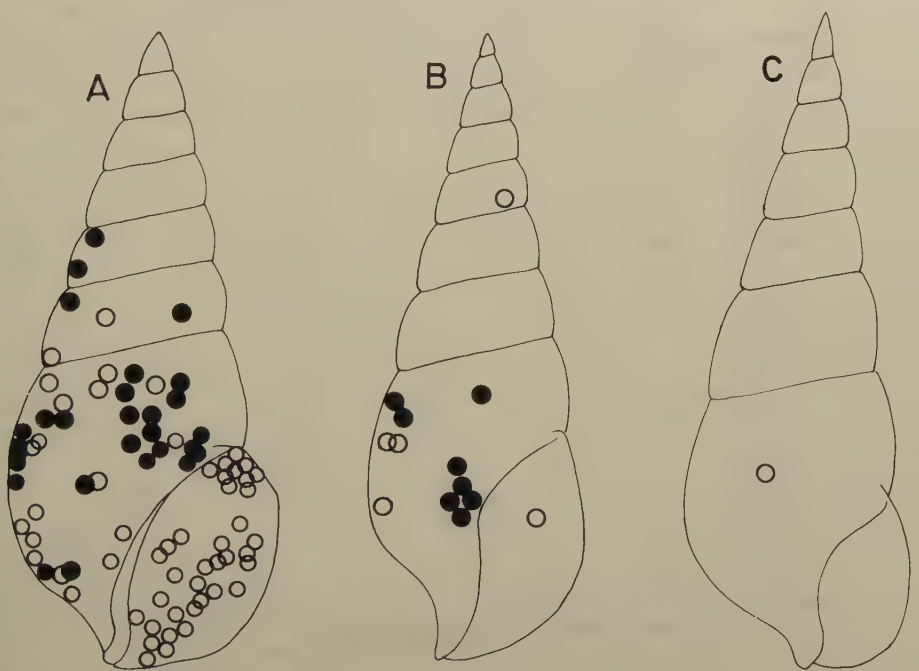


Fig. 6. The position of *Patelloida lampanicola* on *Batillaria zonalis* (A), *B. multiformis* (B), and *B. cumingii* (C): ● represent positions on the ventral surface of the shell; ○ represent positions on the dorsal surface of the shell.

were collected each month. These data are set out in Table II which also suggests that the species of *Batillaria* are colonized in the sequence *B. zonalis* > *B. multiformis* > *B. cumingii* although this may merely reflect relative abundance. Fig. 5 shows the incidence and length of specimens of *Patelloida lampanicola* on *Batillaria* and details of air and sea temperatures, rainfall, and sea salinity over the period studied. The Figure also identifies two very characteristic habits of *Batillaria*. These are "burrowing", at least at low tide when the specimens were collected, and "clustering" when large numbers of the animals congregate together, presumably to breed. There was clustering in February and August 1977. From September to December >50%

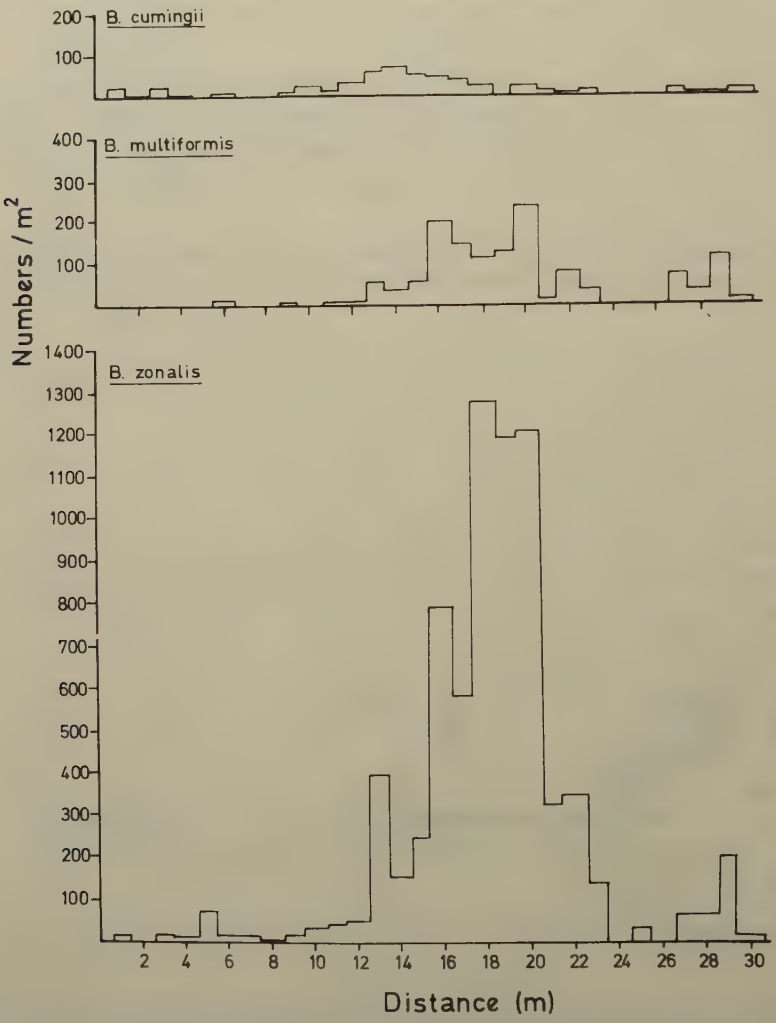


Fig. 7. The zonation of *Batillaria cumingii*, *B. multiformis*, and *B. zonalis* upon the shore at Tai Tam as recorded from transect A–A′ during December 1977: distance = length of A–A′.

of the potamid population was buried. This also happens in the New Zealand mud snail *Amphibola crenata* (Morton, 1975). The records show that during the period when the potamid population is buried very few specimens of *Patelloida lampanicola* were collected from the shells – the limpets possibly migrating onto stones (as will be discussed later). During the "clustering" periods a relatively greater number of specimens of *P. lampanicola* were found. If clustering does represent breeding behaviour in *Batillaria* it seems at least possible that reproduction in *Patelloida lampanicola* is correlated with it to aid species survival.

Burrowing in *Batillaria* is at the times of low winter air and sea temperatures and maximal salinity correlated with low rainfall. Clustering in *Batillaria* (and possibly breeding) coincides with a slow warming of the waters after the winter low in February and the cooling of the waters in August after a summer high.

The position of the limpets on each specimen of *Batillaria* is shown in Fig. 6. It can be seen that the species are colonized in the sequence *B. zonalis* (86%), *B. multiformis* (12%), and *B. cumingii* (1%). The limpets typically attach to the body whorl and are usually found on the back of the snail above the aperture, but are also found adjacent to the aperture. Like *Acmaea asmi* on *Tegula funebralis* (Eikenberry & Wickizer, 1964) they presumably feed on the surface covering of diatoms and other microscopic life that attaches to the shell, much as it would to any solid object in the nutrient-rich sands.

At transect A–A′ where the highest densities of *Patelloida lampanicola* were recorded (Fig. 2C), the analysis of monthly samples of *Batillaria* (Fig. 7) has shown that the three species are zoned down the beach i.e. *B. zonalis* occurs in maximum

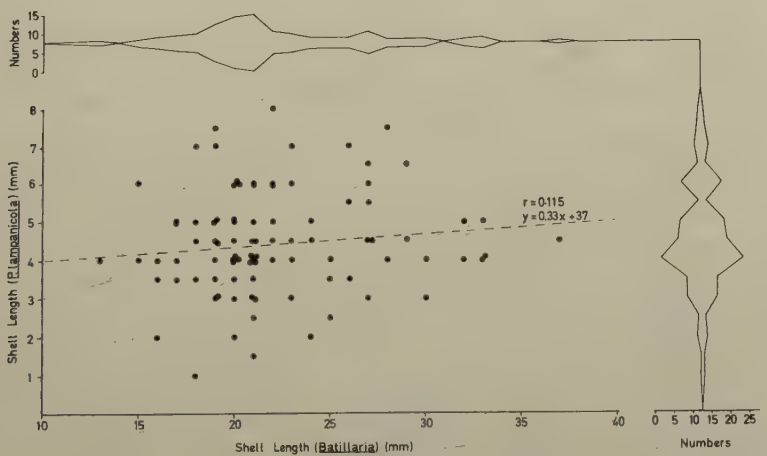


Fig. 8. The shell lengths of a sample of *Patelloida lampanicola* plotted against the shell lengths of the specimens of *Batillaria* upon which they were found: there is no evidence of a linear relationship; the diagram also shows that *Patelloida lampanicola* (4 mm long) most commonly occur on potamids 21 mm long.

numbers lower down the shore than *B. multiformis* which in turn is zoned lower than *B. cumingii*. *Patelloida lampanicola* occurs maximally (Fig. 2C) on the lower reaches of the transect which can be correlated with maximum densities of *Batillaria zonalis* and, to a lesser extent, of *B. multiformis* (Fig. 7). *B. cumingii* is dominant in the more sheltered regions (Transects E–E′ and F–F′) of the sand bars and where, significantly, *Patelloida lampanicola* is rarely found.

Fig. 8 shows that *P. lampanicola* attaches in maximal numbers to *Batillaria* shells ≈ 20–21 mm long. The correlation coefficient ($r$) between length in *Patelloida lampanicola* and *Batillaria* is 0.115 and is not significant. Thus the population of *Patelloida lampanicola* is randomly distributed on the *Batillaria* population (provided they are >13 mm in length). This also lends some support to a contention, as will be discussed later, that the limpets move from snail to snail, as they presumably must in order to obtain sufficient food. From Fig. 9, however, it can be seen

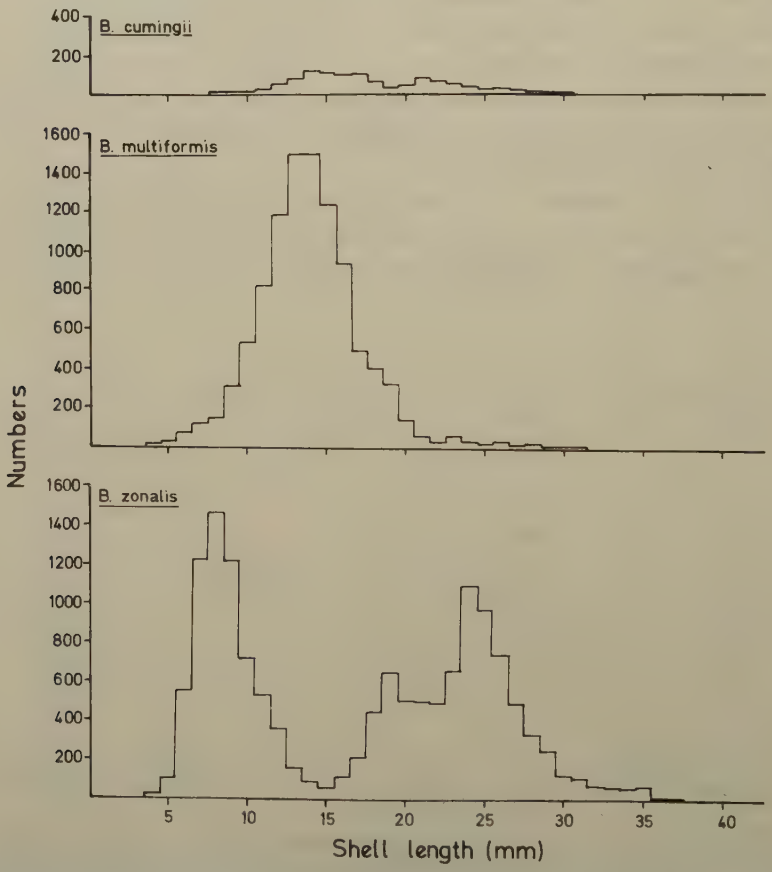


Fig. 9. Length frequency histograms for the populations of *Batillaria cumingii*, *B. multiformis*, and *B. zonalis* from transect A–A′ in December 1977 during a period of no growth.

that *Batillaria* of length 21 mm (i.e. those most often colonized by *Patelloida lampanicola*) are largely *Batillaria zonalis*. Fig. 9 also illustrates that *B. zonalis* is dominant on the lower shore (Fig. 7), especially of transects A–A′, B–B′, and C–C′. Thus, for a number of reasons, *Patelloida lampanicola* typically associates with *Batillaria zonalis*, and to a lesser extent with *B. multiformis*, as will be discussed later.

## CHOICE EXPERIMENTS

The choice experiments substantiate the field observations. In the first experiment (Table IIIA) where limpets of both species were given a choice of *Batillaria* or

TABLE III

The choice experiments: A, 20 each of *P. pygmaea* and *P. lampanicola* given the choice of *Batillaria*, stones, shells or gravel; B, 20 each of *P. pygmaea* and *P. lampanicola* given the choice of 20 specimens each of *B. multiformis*, *B. zonalis*, and *B. cumingii* (of approximately equal size), the dish or gravel – mean results from two trials given; C, 20 each of *P. pygmaea* and *P. lampanicola* given the choice of either the dish, stones, gravel or the tank itself.

| A | *Batillaria* | | Stones | | Shells | | Gravel | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % | Total |
| *P. pygmaea* | 5 | 25 | 10 | 50 | 4 | 20 | 1 | 5 | 20 |
| *P. lampanicola* | 20 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 20 |

| B | | *B. multiformis* | | *B. zonalis* | | *B. cumingii* | | Dish | | Gravel | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % | No. | % | No. | % | Total |
| *P. pygmaea* | Trial 1 | 1 | 5 | 2 | 10 | 0 | 0 | 14 | 70 | 3 | 15 | 20 |
| | Trial 2 | 1 | 5 | 3 | 15 | 1 | 5 | 10 | 50 | 5 | 25 | 20 |
| | Total | 2 | 5 | 5 | 12.5 | 1 | 2.5 | 24 | 60 | 8 | 20 | 40 |
| | Mean | 1 | 5 | 2.5 | 12.5 | 0.5 | 2.5 | 12 | 60 | 4 | 20 | 20 |
| *P. lampanicola* | Trial 1 | 5 | 25 | 7 | 35 | 3 | 15 | 4 | 20 | 1 | 5 | 20 |
| | Trial 2 | 9 | 45 | 7 | 35 | 2 | 10 | 2 | 10 | 0 | 0 | 20 |
| | Total | 14 | 35 | 14 | 35 | 5 | 12.5 | 6 | 15 | 1 | 2.5 | 40 |
| | Mean | 7 | 35 | 7 | 35 | 2.5 | 12.5 | 3 | 15 | 0.5 | 2.5 | 20 |

| C | Stones | | Dish | | Gravel | | Tank | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % | Total |
| *P. pygmaea* | 9 | 45 | 11 | 55 | 0 | 0 | 0 | 0 | 20 |
| *P. lampanicola* | 11 | 55 | 6 | 30 | 2 | 10 | 1 | 5 | 20 |

stones, all specimens (20) of *Patelloida lampanicola* were, highly significantly ($P < 0.005$), recovered from the *Batillaria* after only 2 h. After 2 days the 20 specimens of *Patelloida pygmaea* were distributed in the following manner: *Batillaria* (25%), shells (20%), stones (50%). The preference of *Patelloida pygmaea* for substrata other than *Batillaria* is also highly significant ($P < 0.005$).

The second experiment (Table IIIB) also significantly demonstrated ($P < 0.005$) that *Patelloida lampanicola* has a positive attraction to *Batillaria* with, again very rapidly, a mean of 70% of all *Patelloida lampanicola* migrating to the various species of *Batillaria*. These experiments suggest that *B. zonalis* and *B. multiformis* are preferred to *B. cumingii*. Conversely, a mean of 60% (again significantly, $P < 0.005$) of the specimens of *Patelloida pygmaea* remained attached to the dish, only 20% taking up residence on *Batillaria* and the remaining 20% recovered from the gravel. The two species can also be shown to significantly ($P < 0.005$) differ from each other in their choice of substratum, *Patelloida pygmaea* preferring the dish or gravel and *P. lampanicola* the potamid snails.

The final experiment (Table IIIC) effectively demonstrates that in the absence of *Batillaria* both limpets act in approximately the same way. *Patelloida pygmaea* acted as before, taking up residence on the dish and the stones. *P. lampanicola* followed this pattern, also taking up residence on the dish and, to a lesser extent, on the stones. Both sets of observations are significant ($P < 0.005$).

These experiments (Table III) collectively demonstrate yet another aspect of the behaviour of the two limpets; both are highly mobile and can move over a sandy substratum in search of a final site for habitation. This is clearly much more important for *P. lampanicola* than *P. pygmaea*, because it has to move not only from snail to snail to feed but also from the *Batillaria* population to stones when the snails bury themselves.

## Discussion

Species of limpets are notoriously difficult to differentiate because, being typically intertidal, shell characters are not absolutely reliable indices of relationships. Often shell character studies have been supplemented by an examination of the radula to separate, for example, species of *Acmaea* (Test, 1946) and *Patella* (Evans, 1958) although Dehnel (1978) has had to use a whole array of other criteria to separate three closely similar species of *Collisella*. Rarely, however, have any criteria, other than shell characters, been used to separate limpets from Asia and this is particularly true of the genera *Collisella* and *Patelloida* comprising, supposedly, many small species. Thus, the two limpets discussed here i.e. *P. pygmaea* and *P. lampanicola* were originally described as separate species (Dunker, 1861; Habe, 1944) but they have at various times been considered but junior synonyms of *P. heroldi* and *P. conulus*, respectively. Since both of these species may also be synonymous, a

complex taxonomic knot was tightened when Habe (1971) relegated *P. lampanicola* to a "form" of *P. pygmaea*. Thus, "*P. lampanicola*" was assumed to derive its conical form from a percentage of the total population of *P. pygmaea* residing on *Batillaria* shells. *Patelloida pygmaea* would thus be similar to *Patella* (Moore, 1934), *Acmaea* (Shotwell, 1950), and *Cellana* (Rao & Ganapati, 1970) - variations in form derivable from habitation of extremes of the same niche.

Giesel (1970) has shown that *Acmaea digitalis* has two major shell pattern complexes: one, generally light coloured and striped, and the other, dark coloured and striped. The light limpets inhabit colonies of the white goose-neck barnacle *Pollicipes polymerus* J.B. Sowerby, whereas the darker limpets are found solely on the rock faces of the high intertidal. Young limpets actively migrate to the *Pollicipes* colony at a speed commensurate with their pattern score i.e. light limpets arrive quicker. Artificially removed limpets will also move back onto the barnacles. Giesel (1969) has also shown that individuals residing on the *Pollicipes* are more conical in shell form although there is no difference in radula teeth structure between the two morphs. This study has ascertained, however, that the situation seen in *Acmaea digitalis*, as described by Giesel, is not applicable to *Patelloida pygmaea* and its supposed "form" *P. lampanicola*. Despite obvious similarities with the situation in *Acmaea digitalis* it is believed that *Patelloida lampanicola* is a distinct species spatially separated from *P. pygmaea*.

The hard substratum in an otherwise soft shore environment is colonized by species more reminiscent of open rocky shores. Thus in tropical estuaries mangrove stems are colonized by specific barnacles and the limpet-like bivalve *Enigmonia aenigmatica* (Morton, 1976a). On tropical sandy shores the odd stone occurs with empty shells (particularly of *Saccostrea cucullata*), the latter also attached, in life, to stones and other debris. Other empty shells accumulated by wave action also occur. On such shores, bivalves which project the posterior margins of their valves above ground are colonized by encrusting organisms (barnacles and algae). Thus any relatively stable substratum on an otherwise mobile shore is colonized. By far the most numerous epifaunal colonizers of sandy deposits in S.E. Asia are species of *Batillaria* (Potamididae). Members of the Cerithiidae are more common on muddy, mangrove dominated, shores. Such shells, in enormous numbers, provide a hard substratum that is, at least, potentially available to encrusting organisms.

*Batillaria* is, however, largely only colonized by the limpet *Patelloida lampanicola* which, although belonging to the same subgenus as *P. pygmaea* (i.e. *Chiazacmea*) and being very close in colour patterning, can be separated from the latter on the basis of shell morphometrics and radula structure. Giesel (1969, 1970) could find no differences in radula structure between the two colour morph series of *Acmaea digitalis*. The ecological (and thus taxonomic) picture with regard to the Asian limpets has been blurred, however, by the fact that *Patelloida pygmaea* can also be found on *Batillaria* while *Patelloida lampanicola* can also be found on stones or shells. In the first case, *P. pygmaea* approaches *P. lampanicola* in shell form but its inherent

shell plasticity cannot sufficiently approximate the pattern of growth of *P. lampanicola* and is thus incapable of growing (as does *P. lampanicola*) to match the relatively increasing curvature of the potamid shell. It thus presumably becomes progressively more susceptible to dislodgement and predation. Thus only small specimens of *P. pygmaea* (<4.2 mm in length) are found on *Batillaria*. The occurrence of *Patelloida lampanicola* on stones and empty shells probably results from the potamids' habit of burying themselves in the sand, at least for some time, during the winter months. *P. lampanicola* is, presumably, unable to withstand prolonged burial and has to find temporary refuge on another substratum. The reasons for this activity in *Batillaria* are unknown.

An interesting observation is that *Patelloida lampanicola* seems to be loosely species specific to *Batillaria zonalis* (and to a lesser extent, *B. multiformis*). *B. cumingii* is rarely colonized. This may result from mutually similar environmental optima i.e. *B. zonalis* (and *Patelloida lampanicola*) is zoned lower and (with *Batillaria multiformis*) more readily inhabits exposed regions of the beach. *B. cumingii* prefers more nutrient-rich, sheltered areas. Possibly, however, this may be a more intimate relationship and is worthy of study. In view of the rapidity with which *Patelloida lampanicola* found specimens of *Batillaria* under experimental conditions (i.e. as 20 *Patelloida lampanicola* each found a potamid in <2 h it would seem very clear that the relationship is more than just a casual one. In contrast, *P. pygmaea* significantly prefers stones or shells to *Batillaria*).

It is clear that *Patelloida lampanicola*, because its radula is of the typical patelloid type, is scraping encrusting organisms from the potamid shell. This may help keep the shell free (as it certainly is), from encrusting barnacles and rock oysters. It thus exploits the food resources of the *Batillaria* shell. That this resource is a small one is reflected in the very low incidence of *Patelloida lampanicola* on the potamids (Table II; 0.24% for *Batillaria zonalis*). Possibly the limpet also benefits from the respiratory currents of the *Batillaria*. Moreover, the clustering habit of *Batillaria* during the presumed breeding phases might also aid successful reproduction in *Patelloida lampanicola* – bringing together (albeit but to release gametes into the sea) an otherwise widely dispersed community; this is also thought to be possible for *Capulus ungaricus* inhabiting *Turritella* (Thorson, 1965). Similarly the *Patelloida lampanicola* population may itself be regulated by fluctuations in the population density of *Batillaria*, although as demonstrated here, *Patelloida lampanicola* can retire to other substrata in times of stress.

This study has done no more than raise yet further questions with regard to the relationship between *P. lampanicola* and its potamid "host". Such questions urgently require further study. Until this research is done the association is hard to accurately define because *P. lampanicola* can clearly survive away from its host on shells or stones (at least for a time). The relationship is, however, probably more than facultative epizoitism and indeed approaches true symbiosis – both partners benefiting – the host from a clean shell, the limpet from an exclusive food resource

and enhanced breeding potential by bringing into proximity males and females and thus ensuring the best chance of fertilizing the liberated eggs.

On rocky shores where many limpets co-occur, the habitat is clearly subdivided; the species become zoned and partition the available resources. This has been amply demonstrated for species of *Acmaea* by Haven (1973), for *Collisella* by Dehnel (1978) and for a mixed population of *Acmaea* and *Lottia* by Stimson (1973). The latter defends its territory against the former by shoving it off the rock (Galbraith, 1965).

Branch (1975) has reported that a greater proportion of juvenile *Patella cochlear* occur on other limpets. There are, however, few records of patellacean limpets living in intimate association with other snails. Most significantly, however, Eickenberry & Wickizer (1964) have shown that *Acmaea asmi* lives attached to the intertidal snail *Tegula funebralis*. It feeds on the shell fauna of the snail and, in laboratory studies, transfers faster to living snails than empty or autoclaved shells. This would appear to be very similar to the relationship existing between *Patelloida lampanicola* and species of *Batillaria*. These examples clearly show how the patellacean form is sufficiently plastic to enable a mode of life to be adopted that is more reminiscent of the calyptracean limpets. In this superfamily members often assume a commensal mode of life. Thus, *Capulus spondylicola* attaches to scallops (Habe, 1967), *Hipponix conicus* attaches to *Haliotis rubra* (Hartley, 1958), *Crepidula adunca* lives on *Tegula funebralis* (Putnam, 1964) while a dwarf form of *Capulus ungaricus* is commensal on *Turritella communis* (Thorson, 1965). In all of these animals there are important feeding and reproductive adaptations suiting them to an epizoic or commensal mode of life. Thus *Capulus ungaricus* benefits from the water currents of *Turritella* and steals food from the mantle cavity. Only the former advantage is possibly possessed by *Patelloida lampanicola* and, with a relatively unmodified radula, food collection is by scraping the surface film from the shell of the potamid. When this has been removed another shell must be found explaining their rapid mobility and ability to move over sand. Clearly the ancestral stock of *P. pygmaea* and *P. lampanicola* was forced, through selection, to colonize a wide range of hard substrata on otherwise soft shores. Perhaps the situation seen in *Acmaea digitalis*, as described by Giesel (1969, 1970), can explain the evolution of *Patelloida pygmaea* and *P. lampanicola*. Disruptive selection establishes in the former case a bimodal distribution of colour morphs that is maintained by behaviour polymorphism. Is it possible that time could genetically consolidate this situation ultimately resulting in the ecological picture seen in *P. pygmaea* and *P. lampanicola*, i.e. where site specialization has led, ultimately, into niche segregation? Rarely noted on soft shores but characteristic of other tropical communities, notably coral reefs, this study demonstrates a nice example of niche segregation that can be compared with somewhat more advanced examples from the Mollusca. Thus when the two commensal bivalves *Montacuta substriata* and *M. ferruginosa* occur together on their mutual host *Spatangus purpureus*, the former attaches to the ventral spines, the latter to the dorsal spines (Marshall, 1891). Similarly the parasitic eulimid gastropods *Balcis shaplandi* and

*Mucronalia fulvescens* attach respectively, to the aboral and oral surfaces of their mutual host starfish *Archaster typicus* (Morton, 1976b). These examples and the limpets here studied help us to better understand the mechanisms of evolution at a diversity of levels in a diversity of habitats.

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## References

Branch, G. M., 1975. Intraspecific competition in *Patella cochlear* Born. *J. Anim. Ecol.*, Vol. 44, pp. 263–282.

Christiaens, J., 1975a. Revision provisoire des Mollusques marins recents de la famille des Acmaeidae et description de deux nouveaux sub-genera: *Simplacmaea* nov. subgen. et *Collisellacmaea* nov. subgen. *Inf. Soc. belge Malacol.*, Serie 4, No. 1, pp. 3–18.

Christiaens, J., 1975b. Revision provisoire des Mollisques marins recents de la famille des Acmaeidae (second partie). *Inf. Soc. belge Malacol.*, Serie 4, No. 4, pp. 91–116.

Christiaens, J., 1976a. De familie Acmaeidae. *Gloria Maris*, Vol. 15, pp. 49–64.

Christiaens, J., 1976b. The family Acmaeidae-first addendum. *Gloria Maris*, Vol. 15, pp. 127–132.

Dehnel, P. A., 1978. An analysis of morphological variation in limpets of the genus *Collisella*. *Can. J. Zool.*, Vol. 56, pp. 643–650.

Dunker, G. 1861. *Mollusca Japonica*. E. Schweizebart, Stuttgartiae, 36 pp.

Eikenberry, A. B. & D. E. Wickizer, 1964. Studies on the commensal limpet *Acmaea asmi* in relation to its host *Tegula funebralis* (Mollusca: Gastropoda). *Veliger*, Vol. 6 (suppl.), pp. 66–77.

Evans, R.G., 1958. The genus *Patella* on the west coast of France. *J. Conch., Paris*, Vol. 98, pp. 126–151.

Galbraith, R., 1965. Homing behaviour in the limpets *Acmaea digitalis* and *Lottia gigantea*. *Am. Midl. Nat.*, Vol. 74, pp. 245–246.

Giesel, J. T., 1969. Growth and relative growth in the limpet *Acmaea digitalis* Eschscholtz. *Ecology*, Vol. 50, pp. 1084–1087.

Giesel, J. T., 1970. On the maintenance of a shell pattern and behaviour polymorphism in *Acmaea digitalis*, a limpet. *Evolution*, Vol. 24, pp. 98–119.

Habe, T., 1944. On the Japanese Lottiidae (= Acmaeidae). *Venus*, Vol. 13, pp. 171–187.

Habe, T., 1967. A new capulid snail, *Capulus spondylicola*, from Japan. *Venus*, Vol. 26, pp. 37–38.

Habe, T., 1971. *Coloured illustrations of the shells of Japan* (II). Hoikusha Publishing Co. Ltd, Osaka, Japan, 182 pp.

Hartley, T., 1958. Movement by commensal *Hipponix conicus* (Schumacher). *J. malac. Soc. Aust.*, Vol. 2, pp. 37–38.

Haven, S. B., 1973. Competition for food between the intertidal gastropods *Acmaea scabra* and *Acmaea digitalis*. *Ecology*, Vol. 54, pp. 143–151.

Kuroda, T., T. Habe & K. Oyama, 1971. *The sea shells of Sagami Bay*. Maruzen Co., Tokyo, 769 pp.

Lischke, C. E., 1869. *Japanische Meers Conchylien*. Suppl. IV, I Theil. Thedor Fischer, Cassel, 192 pp.

Lischke, D. E., 1871. *Japanische Meers Conchylien*. Suppl. IV, II Theil. Theodor Fischer, Cassel, 184 pp.

Marshall, J. T., 1891. The habitat of *Montacuta ferruginosa*. *J. Conch.*, Vol. 6, pp. 399–404.

MOORE, H. B., 1934. The relation of shell growth to environment in *Patella vulgata*. *Proc. malac. Soc. Lond.*, Vol. 21, pp. 217–222.

MORTON, B., 1975. The seasonal variation in the feeding and digestive cycle of *Amphibola crenata* (Martyn 1784) (Gastropoda: Pulmonata). *Forma Functio*, Vol. 8, pp. 175–190.

MORTON, B., 1976a. The biology, ecology and functional aspects of the organs of feeding and digestion in the S.E. Asian mangrove bivalve, *Enigmonia aenigmatica* (Mollusca: Anomiacea). *J. Zool.*, Vol. 179, pp. 347–466.

MORTON, B., 1976b. Selective site segregation in *Balcis shaplandi* and *Mucronalia fulvescens* (Mollusca: Gastropoda: Aglossa) parasitic upon *Archaster typicus* (Echinodermata: Asteroidea). *Malacol. Rev.*, Vol. 9, pp. 55–61.

OLIVER, W. R. B., 1926. Australasian Patellidae. *Trans. N.Z. Inst.*, Vol. 56, pp. 547–582.

PILSBRY, H. A., 1891. Patellidae, structure and systematics. In, *Manual of conchology, Tome 13*, edited by G. W. Tryon, Acad. nat. Sci., Philadelphia, 195 pp.

PILSBRY, H. A. 1895. Catalogue of marine mollusks of Japan. Frederick Stearns, Detroit, 196 pp.

PUTNAM, D. A., 1964. The dispersal of young of the commensal gastropod *Crepidula adunca* from its host, *Tegula funebralis*. *Veliger*, Vol. 6 (suppl.), pp. 63–66.

RAO, M. B. & P. N. GANAPATI, 1970. Ecological studies on a tropical limpet, *Cellana radiata*. Structural variations on the shell in relation to distribution. *Mar. Biol.*, Vol. 10, pp. 236–243.

RUNHAM, N. W. & P. R. THORNTON, 1967. Mechanical wear of the gastropod radula: a scanning electron microscope study. *J. Zool.*, Vol. 153, pp. 445–452.

SHOTWELL, J. A., 1950. Distribution of volume and relative linear measurement changes in *Acmaea*, the limpet. *Ecology*, Vol. 31, pp. 51–61.

SOKAL, R. R. & J. ROHLF, 1969. *Biometry*. W. H. Freeman & Co., San Francisco, 776 pp.

STIMSON, J., 1973. The role of territory in the ecology of the intertidal limpet *Lottia gigantea* (Gray). *Ecology*, Vol. 54, pp. 1020–1030.

TEST, A. R., 1946. Speciation in limpets of the genus *Acmaea*. *Contr. Lab. vertebr. Biol. Univ. Mich.*, Vol. 31, pp. 1–24.

THORSON, G., 1965. A neotenous dwarf-form of *Capulus ungaricus* (L.) (Gastropoda, Prosobranchia) commensalistic on *Turritella communis* Risso. *Ophelia*, Vol. 2, pp. 175–210.

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# A POSTERIORLY BIFID SPECIMEN OF THE ANTARCTIC HETERONEMERTEAN *PARBORLASIA CORRUGATUS* (McIntosh, 1876)

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**Abstract:** A posteriorly bifid example of the Antarctic heteronemertean *Parborlasia corrugatus* (McIntosh, 1876) is described and illustrated. Histological studies reveal that in the basal portion of one tail fork the body wall muscles and intestinal diverticula are less well developed than in other regions, and that gonads are missing. It is concluded that the monstrosity is the result of aberrant regeneration subsequent to the animal receiving a deep injury.

## INTRODUCTION

Although the duplication of body parts in nemerteans is by no means unusual during experiments on regeneration (e.g., see Coe, 1930), such "Individuals ... must be considered as monstrosities, with fewer chances of survival than normal individuals" (Coe, 1943, see p. 196). Where duplication does take place it is most commonly restricted to specific internal organs, such as the proboscis, accessory stylet pouches or statocysts (see, e.g., Coe, 1943; Kirsteuer, 1977), and is not externally evident. There are few records of naturally occurring nemerteans in which the head or tail is duplicated or a lateral branch has developed with a complete set of contained organs, and all are restricted to the order Heteronemertea. Examples have been found in *Lineus sanguineus* (Rathke, 1799) (McIntosh, 1873–74; Gontcharoff, 1949), *Baseodiscus delineatus* (Delle Chiaje, 1825) (Bürger, 1897–1907) and *Cerebratulus marginatus* Renier, 1840 (McIntosh, 1906); no comparable monstrosity has previously been described for *Parborlasia corrugatus*, despite the species being one of the most commonly recorded Antarctic nemerteans.

## MATERIAL AND METHODS

The present specimen was one of 32 examples of the species collected with a Blake trawl by the United States Antarctic Research Program from 20–27 m depth on 16 March 1970. The trawl area extended from 63°30′03″W; 63°30′15″W longitude along latitude 64°49′13″S. The nemertean, originally preserved in alcohol, was

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sectioned at 6–7 $\mu$m in 56 °C m.pt paraffin wax. Sections were exposed to a saturated alcoholic picric acid solution for 10 min prior to staining with Mallory's trichrome; this procedure has been found to enhance the colour differentiation of Mallory in nemertean sections compared with that obtained with the direct staining of alcohol-preserved material.

## Results

### External features

The external appearance of the specimen is shown in Fig. 1. The animal was 11 cm long, 1.3 cm in maximum width and up to 0.8 cm in dorsoventral thickness.

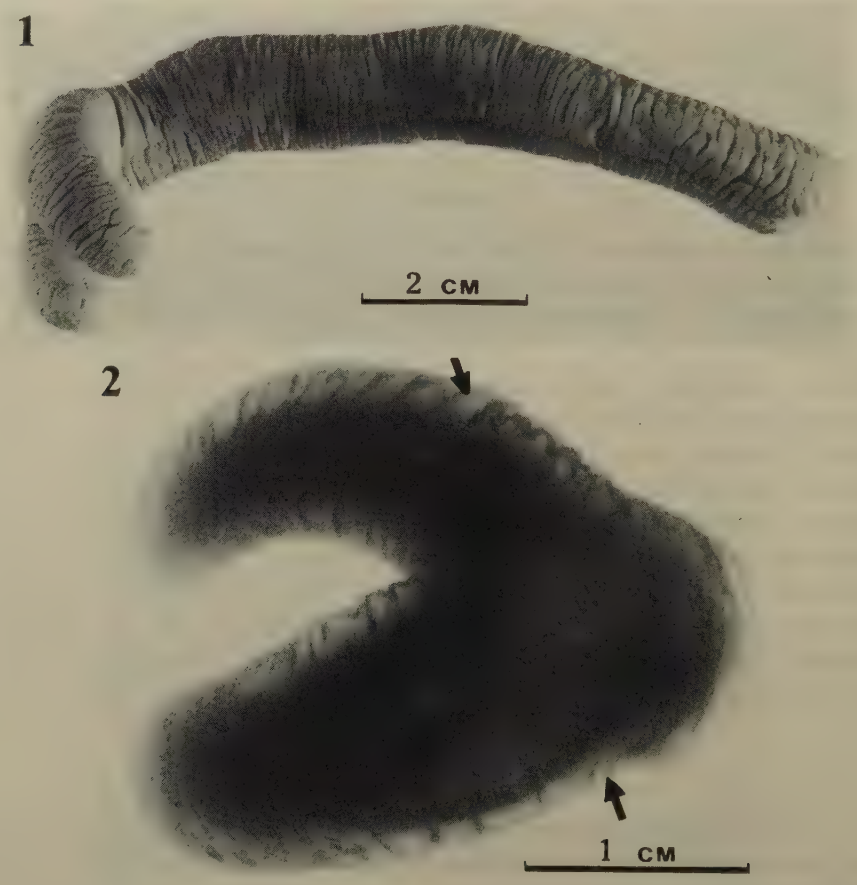


Fig. 1. Intact specimen of *Parborlasia corrugatus* with twinned tails, viewed from right ventrolateral aspect.

Fig. 2. Dorsal view of the bifid posterior end after clearing in xylene: note the unequal sizes of the two tails and the clearer area in the basal regions of the right branch; the arrows indicate the approximate plane of sectioning of Fig. 3.

Apart from the twinned posterior tip it has the typical appearance of a preserved *P. corrugatus*.

The unequal lengths and widths of the two tails (Fig. 2) suggest that one is a regenerated portion, whilst the other represents the original posterior region of the nemertean. In the cleared specimen the pale area near the base of the larger tail (Fig. 2) shows the limits of reduced anatomical development described below.

## INTERNAL ANATOMY

The internal anatomy of *P. corrugatus* has been described on several occasions, most recently by Friedrich (1970), and the structure of the present specimen is in agreement with previous accounts. Accordingly it is only with the histology of the tail branches that the present paper is concerned.

Both branches contain a full complement of body parts, including the blood system, nervous system, alimentary tract, rhynchocoel, and gonads. Differences can be distinguished, however, in the degree of development of particular components, especially near the point of bifurcation (Fig. 3).

The outer body wall layers (epidermis, subepidermal musculature, and dermis) are essentially the same in both tails and no significant differences can be distinguished. The three main body wall muscle layers, however, are not as well developed in the anterior portions of the right branch when compared with those of the unbranched trunk and the left fork. Reduced development is apparent in the density and arrangement of the muscle fibres comprising each layer; those of the outer longitudinal zone especially are more haphazardly distributed rather than arranged into radially orientated muscle blocks, and the individual bundles of fibres are often separated from each other by parenchymatous connective tissues rather than lying closely appressed to their neighbours. The muscle strata are also reduced in overall thickness to an extent which is unlikely to be accounted for merely by local variations in body contraction. Minimum widths of the three muscle layers of the right tail fork, compared with those measured for the left branch (listed in parentheses) are 20 $\mu$m (150 $\mu$m) for the inner longitudinal zone, 90 $\mu$m (180 $\mu$m) for the circular musculature, and 150 $\mu$m (300 $\mu$m) for the outer longitudinal layer.

A striking difference is also found between the development of the intestinal diverticula in the anterior portion of the right tail and that occurring in the remaining intestinal regions of the body (Fig. 3). Their gastrodermal lining is very much reduced in height (7–45 $\mu$m compared with 15–100 $\mu$m or more in, for example, the left fork) and, as judged by the greatly reduced gland cell frequency and density of food vacuoles in the ciliated interstitial cells, it is not as physiologically active. Interdiverticular muscle fibres too are much more weakly formed than elsewhere. The diverticula in general present a "septate" appearance.

One further feature of the right tail is that, where it joins the undivided trunk,

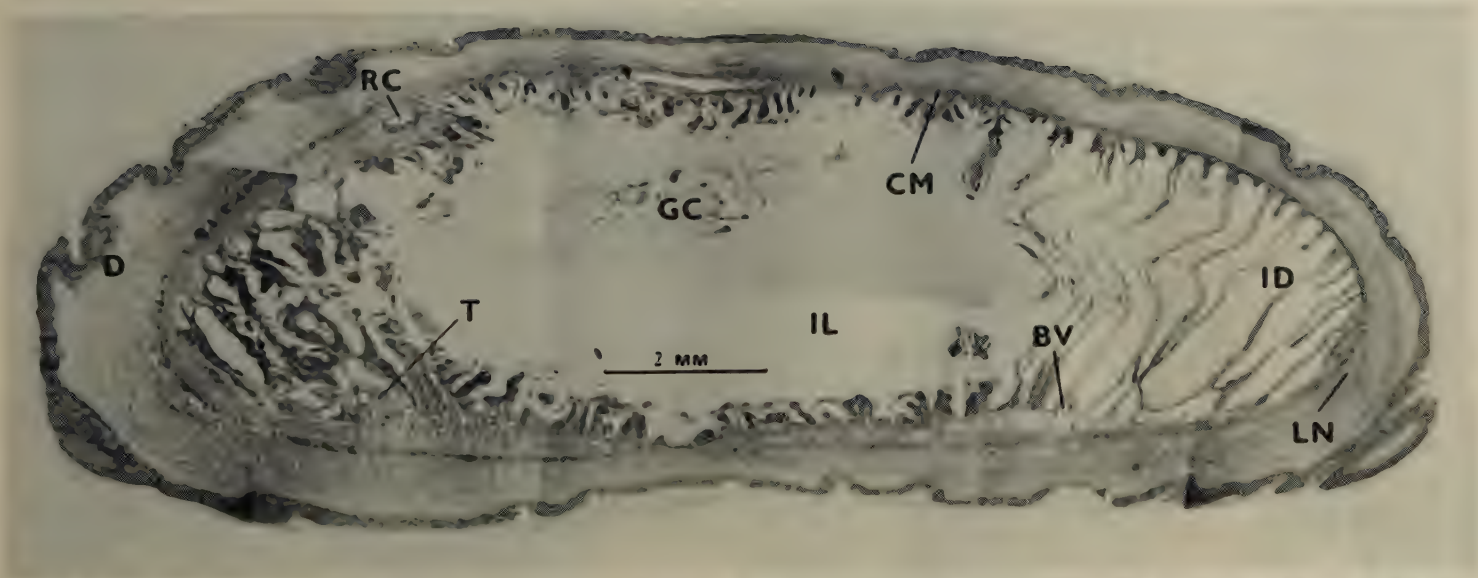


Fig. 3. Photomicrographic montage through the posterior body to show the different appearance of structures on either flank. BV, blood vessel; CM, body wall circular muscle layer; D, dermis; GC, gut contents; ID, intestinal diverticula; IL, intestinal lumen; LN, lateral nerve cord; RC, rhynchocoel; T, testis. Mallory stain.

it contains no gonads. In other parts of the intestinal region mature testes, containing spermatids and spermatozoa, are distributed between the diverticula.

The anatomical changes described above are only apparent in the anterior region of the right fork. Further back, away from the point of bifurcation, the tissues and organs progressively develop an appearance like those in other parts of the body. In the posterior regions no histological differences can be distinguished between the two tails.

## Discussion

Coe (1943) comments that although the duplication of body parts in nemerteans may result either from the partial fusion of two developing ova or by the division of an embryonic blastema into two portions, it more often occurs during the regenerative processes which follow an injury. It is clearly the latter case which applies to this specimen of *P. corrugatus* since evidence of an original injury, whatever its cause, is provided by the reduced or modified development of the body wall musculature, intestinal diverticula and gonads in the basal region of the right fork. In his analysis of nemertean regenerative processes Coe (1934, see p. 314) concludes that "cut nerve cords liberate an agent which activates the dormant cells of the parenchyma and transforms them into regenerative cells". It thus appears probable that the Antarctic specimen suffered an injury deep enough to damage the lateral nerve cords but that the subsequent regenerative mechanisms, instead of merely healing the wound, developed in an aberrant fashion and produced a second tail. What is unusual, however, is that despite the complete regeneration of posterior body parts the tissues around the point of damage have remained hypoplastic.

## Acknowledgements

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## References

Bürger, O., 1897–1907. Nemertini (Schnurwürmer). In, *Klassen und Ordnungen des Tier-Reichs, Vol. 4, Supplement*, edited by H. G. Bronn and C.F. Winter, Leipzig, 542 pp.

Coe, W. R., 1930. Regeneration in nemerteans. II. Regeneration of small sections of the body split or partially split longitudinally. *J. exp. Zool.*, Vol. 57, pp. 109–144.

Coe, W. R., 1934. Analysis of the regenerative processes in nemerteans. *Biol. Bull. mar. biol. Lab., Woods Hole*, Vol. 66, pp. 304–315.

Coe, W. R., 1943. Biology of the nemerteans of the Atlantic coast of North America. *Trans. Conn. Acad. Arts Sci.*, Vol. 35, pp. 129–328.

FRIEDRICH, H., 1970. Nemertinen aus Chile. *Sarsia*, No. 40, pp. 1–80.

GONTCHAROFF, M., 1949. Sur un cas de régénération anormale chez la Némerte *Lineus ruber β*. *C. r. hebd. Séanc. Acad. Sci., Paris*, Vol. 228, pp. 1757–1758.

KIRSTEUFR, E., 1977. Remarks on taxonomy and geographic distribution of the genus *Ototyphlonemertes* Diesing (Nemertina, Monostilifera). *Mikrofauna Meeresboden*, Vol. 61, pp. 167–181.

McINTOSH, W. C., 1873–74. *A monograph of the British annelids. Part 1. The nemerteans*. Ray Society Publs, London, 214 pp.

McINTOSH, W. C., 1906. On bifid nemerteans (*Cerebratulus angulatus*, O.F.M., = *marginatus*, Renier?) from Aberdeen and Naples. *Ann. Mag. nat. Hist., Ser. 7*, Vol. 17, pp. 74–78.

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# DEFENSIVE RESPONSES OF GASTROPODS TO THE PREDATORY FLATWORMS *FREEMANIA LITORICOLA* (Heath and McGregor) AND *NOTOPLANA ACTICOLA* (Boone)

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**Abstract:** Three species of gastropods were tested in the laboratory and in the field for responses to contact with the polyclad flatworms *Freemania litoricola* (Heath and McGregor) and *Notoplana acticola* (Boone). The three limpets differed considerably in their responses to *Freemania litoricola*. All specimens of *Notoacmea scutum* (Rathke) responded in some way to this flatworm, mostly by running. Fewer than half of the *Collisella digitalis* (Rathke) responded to *Freemania litoricola*, and these responded by mushrooming – elevating the shell several millimeters off the substratum. *Collisella scabra* (Gould) did not respond any differently to contact with *Freemania litoricola* than to contact with a control probe. Differences in the responses of these limpets to *Freemania litoricola* are discussed in relation to the characteristic intertidal distributions of this flatworm and the limpets. In contrast to *Freemania litoricola*, the polyclad *Notoplana acticola* typically did not elicit defensive responses. This difference is related to the diets of the two flatworms. *Freemania litoricola* was found to be a predator on limpets, whereas no evidence was found that *Notoplana acticola* eats limpets.

## INTRODUCTION

Defensive responses of marine invertebrates to predators are common, and examples of responsive prey span a wide variety of taxa (reviews by Ansell, 1969; Feder, 1972; Mackie & Grant, 1974). However, the predators that have been shown to trigger these responses almost exclusively belong to two taxa, the asteroid echinoderms and the gastropod molluscs. Here we report the first examples of defensive responses triggered by a marine polyclad flatworm. One other example has been observed in the terrestrial environment. Froehlich (1955) described defensive behavior by a slug in response to land planarians; the response consisted of discharge of mucus and accelerated locomotion.

*Freemania litoricola* and *Notoplana acticola* are common polyclad flatworms in rocky intertidal regions along the Pacific coast of North America (Freeman, 1933; Hyman, 1953). *Freemania litoricola* is found from Monterey Bay, California, to British Columbia, and *Notoplana acticola* occurs all along the coast of California

(Hyman, 1953). In California, Heath & McGregor (1912) found specimens of *Freemania litoricola* in Monterey Bay in mid and low intertidal areas, whereas near Bodega Bay, we commonly observed this species extending up to about the mean lower-high water mark. Similarly, Thum (1974) found *Notoplana acticola* to be common in low as well as in high intertidal areas at Bodega Bay.

The natural diets of *Freemania litoricola* and *Notoplana acticola* are not known, but most polyclads are thought to be predators or carnivores (e.g., MacGinitie & MacGinitie, 1968). One polyclad, *Alloioplana californica* (Heath and McGregor), is definitely known to eat gastropods (Heath & McGregor, 1912). In the laboratory, specimens of *Notoplana acticola* have been fed the crustacean *Artemia salina* (L.) by Koopowitz *et al.* (1976) and the small red nudibranch *Rostanga pulchra* MacFarland by Boone (1929). Neither of these laboratory prey, however, are likely to contribute significantly to this flatworm's natural diet.

Among the common mobile gastropods along the Pacific coast of North America are the limpets *Notoacmea scutum* (Rathke), *Collisella digitalis* (Rathke), and *Collisella scabra* (Gould). *Notoacmea scutum* characteristically occurs in mid and low intertidal regions, whereas *Collisella digitalis* and *C. scabra* generally occur much higher (Ricketts *et al.*, 1968). The broad vertical distributions of both *Freemania litoricola* and *Notoplana acticola* overlap those of all three species of limpets.

## Materials and Methods

The flatworms *Freemania litoricola* (Heath and McGregor) and *Notoplana acticola* (Boone) were collected during lower-low water at several sites along the Sonoma County coast of California. Specimens of *Freemania litoricola* (hereafter *Freemania*) were collected at night as they glided across the surface of large boulders. Often they were associated with the limpets *Notoacmea scutum* and *Collisella digitalis*, but occasionally they were found near beds of the mussel *Mytilus californianus* Conrad. Specimens of *Notoplana acticola* (hereafter *Notoplana*) were collected during the day by turning over rocks in channels and tidepools.

Immediately after collection, flatworms were placed in plastic vials in an ice-chest for transport to the laboratory. There, they were kept in separate plastic jars to prevent them from attacking each other. Each jar was fitted with a screen cover, and all were submerged in a refrigerated, recirculating aquarium. Due to high mortality, several collections of both species were necessary.

The limpets *Notoacmea scutum, Collisella digitalis,* and *C. scabra* were collected from rock walls during low tides and transported to the laboratory in ice-chests. Limpets were transferred to the glass face of a recirculating aquarium and left undisturbed for at least 8 h before testing.

Sets of 25 limpets were tested for responses to contact with *Freemania, Notoplana*, and a control probe that consisted of a thin, oval-shaped piece of nylon netting.

To initiate a trial, a flatworm or the control probe was brought gently into contact with a section of the relaxed limpet's foot and mantle margin. Contact was maintained for 30 s while observations were made. Observations were continued for an additional 30 s after contact was stopped. At the end of each trial, the distance traveled by the limpet during the 60-s period was measured, and the limpet's shell length recorded. All tests were conducted under dim light (< 50 lux) due to the pronounced photonegativity of the flatworms, and to the limpets' tendency to raise their shells higher under these conditions. The slightly raised shell made it possible to see that contact between the flatworm and the soft parts of the limpet had been made. A given flatworm was used for no more than 10 trials.

## Results

### *Notoacmea scutum*

The responses of *N. scutum* to contact with a control probe, *Notoplana*, and *Freemania* are summarized in Table I. When contacted by the control probe, most of the animals (21 of 25) withdrew the part of the mantle margin near the point of contact and also retracted their cephalic tentacles. This brief set of reactions to the control was defined as "no response". Four of the 25 animals moved 1 cm or more away from their original location, and these were scored as having given a running response to the control.

The general response of *Notoacmea scutum* to the polyclad *Notoplana* was similar to that given to the control probe. Twenty-three of the 25 animals gave no response. Two gave positive responses: one by running and one by elevating its shell several millimeters off the substratum, a response aptly described as mushrooming by Bullock (1953). The difference between the number of positive responses (running plus mushrooming) to the control probe and to *Notoplana* was not statistically significant ($\chi^2$ test; $P > 0.5$).

In contrast to their general lack of response upon contact with the control probe or *Notoplana*, all of the 25 limpets reacted vigorously to contact with *Freemania*. Immediately, the cephalic tentacles and mantle margin were withdrawn under the shell, and in 24 of the 25 trials, the animals started to run (19 trials) or to mushroom (5 trials). During these running and mushrooming responses, the cephalic tentacles were extended far from the body and waved in all directions. Whereas simple contact with *Freemania* was sufficient to trigger running and mushrooming behavior, the continued presence of the flatworm on the limpet's shell invariably led to a vigorous running response. Limpets continued to run for $\approx 1$ min after last contact with the flatworm, and 14 of the 19 running animals moved up the wall of the aquarium after their initial response (binomial test, $P = 0.032$). No significant difference was found between the sizes of limpets that ran and those that

TABLE I

Responses of three species of limpets to contact with a control probe and the polyclad flatworms *Notoplana* and *Freemania*; $N = 25$.

| | *Notoacmea scutum* | | | *Collisella digitalis* | | | *Collisella scabra* | | |
|---|---|---|---|---|---|---|---|---|---|
| Stimulus | Run | Mushroom | No response | Run | Mushroom | No response | Run | Mushroom | No response |
| Control | 4 | 0 | 21 | 0 | 0 | 25 | 0 | 0 | 25 |
| *Notoplana* | 1 | 1 | 23 | 1 | 3 | 21 | 0 | 1 | 24 |
| *Freemania* | 19 | 5 | 0* | 0 | 11 | 14 | 0 | 1 | 24 |

* One specimen of *N. scutum* clamped down its shell in response to *Freemania*.

mushroomed ($t$-test; $P > 0.2$); similarly no correlation was found between the size of the limpets and the distance traveled during the observation period.

The one limpet that did not run or mushroom upon contact with *Freemania* clamped its shell down on the substratum. This was considered a positive response to *Freemania* (Table I) because nothing similar was seen in response to *Notoplana* or to the control, and also because of the immediate consequence of this behavior. When the limpet clamped down, the flatworm was caught under the limpet's shell and suffered considerable tissue damage.

The combined results indicate that *Notoacmea scutum* responded vigorously and quite specifically to *Freemania*. The difference between the number of positive responses to *Freemania* and *Notoplana* was highly significant ($\chi^2$ test; $P < 0.001$) as was the difference between *Freemania* and the control ($\chi^2$ test; $P < 0.001$). Furthermore, the few running responses that were recorded in response to the control were clearly less vigorous than those elicited by *Freemania*: the distances traveled by moving limpets touched by the control ($\bar{X} = 2.1$ cm) and *Freemania* ($\bar{X} = 6.0$ cm) were significantly different (Median test; $P < 0.05$).

### *COLLISELLA DIGITALIS*

When specimens of *C. digitalis* were touched by the control probe, the mantle and cephalic tentacles were withdrawn briefly, and then re-extended to their resting position. No running or mushrooming was observed (Table I). Similarly, most (21 of 25) *C. digitalis* gave no response to contact with *Notoplana*. One moved a short distance (1.5 cm), and three displayed mushrooming responses.

In contrast, when contacted by *Freemania*, 11 of the 25 limpets responded with pronounced mushrooming. Upon initial contact, these limpets withdrew their tentacles under the shell. Seconds later, the shell was elevated far off the substratum and tilted backwards. The limpets remained in this position for at least 60 s after contact with the flatworm stopped. While mushrooming, the cephalic tentacles of *Collisella digitalis* remained retracted, unlike their extended position in *Notoacmea scutum*. No running responses were observed, and 14 of the 25 limpets did not react differently to *Freemania* than to the control. However, since no mushrooming responses by *Collisella digitalis* occurred in the control, the positive responses to *Freemania* are highly significant ($\chi^2$ test; $P < 0.001$). A few positive responses were triggered by *Notoplana*. The difference between the responses to *Freemania* and *Notoplana* was narrowly insignificant ($\chi^2$ test; $0.05 < P < 0.1$), suggesting incomplete specificity.

### *COLLISELLA SCABRA*

Specimens of *C. scabra* responded to contact with the control by withdrawing part of the mantle and their cephalic tentacles under the shell. This was followed

by the slow lowering of the shell nearest the point of contact. If contact was repeated on the opposite side of the body, the limpet readjusted its shell and lowered it again on the stimulated side.

In almost all trials, contact with *Notoplana* and *Freemania* elicited behavior similar to that in the control. For both species, 24 individuals gave no response, and only one gave a weak mushrooming response. Even repeated and prolonged contacts between *Freemania* and *Collisella scabra* usually did not trigger mushrooming, and running was never observed (Table I).

In summary, the three species of limpets differed qualitatively and quantitatively in their characteristic responses to contact with the flatworm *Freemania*. *Notoacmea scutum* responded most often by running, and its responses were the most vigorous of the three. *Collisella digitalis* responded, if at all, by mushrooming, and not by moving. Significantly fewer *C. digitalis* responded positively than did *Notoacmea scutum* ($\chi^2$ test; $P < 0.001$). *Collisella scabra* did not respond any differently to *Freemania* than it did to the control. The responses of the three to contact with the control probe differed little, and most specimens of all three species reacted to contact with the flatworm *Notoplana* as they did to the control.

### FIELD OBSERVATIONS

Observations in the field yielded information regarding the interaction between *Freemania* and the limpets *Notoacmea scutum* and *Collisella digitalis*. During low tides at night or very early in the morning, specimens of *Freemania* were observed gliding over the surface of exposed, damp rocks to which the limpets *Notoacmea scutum* and *Collisella digitalis* were attached. When a moving flatworm contacted an immobile specimen of *Notoacmea scutum*, the limpet reacted as it did in the laboratory; it immediately elevated its shell and began to run across the exposed rock. This flight behavior of *N. scutum* was observed repeatedly in the field. In contrast, when a flatworm contacted a *Collisella digitalis*, the limpet seemed to respond little if at all to its presence.

*Freemania* collected for experiments often had partially digested limpets in their gut, but the limpet's shell was absent, precluding field identification of the prey species. On two occasions in the field, flatworms were observed in the process of consuming limpets known to be *Collisella digitalis*, and on one occasion, a small specimen of *Notoacmea scutum*.

## Discussion

The defensive responses of *N. scutum* to the predatory polyclad *Freemania* are similar in most respects to this limpet's vigorous responses to predatory seastars (Bullock, 1953; Feder, 1963; Margolin, 1964b; Phillips, 1975). The most frequent response to contact with *Freemania* is rapid movement away from the point of

contact, often followed by movement up a vertical surface. Less frequently, the limpet responds by mushrooming – raising the visceral mass several millimeters off the substratum.

Although the limpets' defensive responses to seastars and flatworms are similar, there are two notable differences, one in context and one in form. The difference in context is that flight from seastars almost always occurs when the limpet is under water, whereas flight from *Freemania* may occur as frequently when the limpet is out of water as under water. The difference in form is that occasionally a limpet will clamp its shell down on the substratum instead of fleeing upon first contact. Flatworms caught under the shell when the limpet clamped down suffered considerable damage. Such clamping is a common response of limpets to contact with inanimate objects, but there is no tendency, even transitory, for limpets to clamp down in response to contact with seastars (Bullock, 1953). Although clamping in response to *Freemania* was only observed once in the 25 trials reported in Table I, it was observed on several additional occasions during preliminary trials and in the field. Branch (1979) has reported that larger specimens of the limpets *Patella oculus* and *P. granatina* regularly lift their shell in response to predatory seastars and gastropods, and then aggressively clamp it down on the predator.

While contact with *Freemania* consistently elicited vigorous responses by *Notoacmea scutum*, similar contact with *Notoplana* rarely did so. Our observations indicate that limpets form at least a part of the natural diet of *Freemania* in central California. The natural diet of *Notoplana* is still unknown, but it seems unlikely to include significant numbers of highly mobile gastropods such as *Notoacmea scutum; Notoplana* was never observed attacking limpets in the field or in the laboratory. Several gastropods, including *Notoacmea scutum*, have previously been shown to respond specifically to seastar predators while ignoring non-predators (Edwards, 1969; Phillips, 1976, 1977). We suggest the responses of *N. scutum* to the flatworms *Freemania* and *Notoplana* also display this specificity.

*Collisella digitalis* and *C. scabra*, in contrast to *Notoacmea scutum*, do not flee from *Freemania*; nor do they flee when touched by predatory seastars (Bullock, 1953; Feder, 1963; Margolin, 1964b). However, *Collisella digitalis* is not totally unresponsive to contact with *Freemania*. In almost half of the trials, *Collisella digitalis* responded by mushrooming when contacted by *Freemania*, whereas this limpet rarely did so when contacted by *Notoplana*, and never did so when contacted by the control probe. Thus, *Collisella digitalis* seemed capable of distinguishing between contact with *Freemania* and contact with some neutral objects. Although contact with the predator frequently triggered mushrooming, the defensive value of this response, if any, is uncertain. We suggest two possible benefits of this behavior, although neither is totally acceptable. First, Margolin (1964a) noted that predatory seastars seemed to be repelled by isolated mantle tissue of the limpet *Diodora aspera*, and the mushrooming response of *Collisella digitalis* would

expose more of the secretory mantle and foot toward the predator. The predator may find these tissues repellent, or the tissues may produce additional mucus to counteract the adhesive glands of the predator. Secondly, mushrooming increases the profile area of the limpet, and may make it too large or more difficult to consume.

The difference in responsiveness between the highly responsive *Notoacmea scutum* and the less responsive *Collisella digitalis* and *C. scabra* undoubtedly relates to the intertidal distributions of *Freemania* and these limpets. *Freemania* and *Notoacmea scutum* are most commonly found below mean tide level (Heath & McGregor, 1912; Ricketts *et al.*, 1968). Since they coexist throughout the mid and low intertidal regions, the number of encounters between predator and prey would be expected to be high, promoting the development of effective defenses by the prey. In contrast, *Collisella digitalis* and *C. scabra* characteristically live high in the intertidal region, and whereas the vertical distributions of *Freemania*, *Collisella digitalis*, and *C. scabra* do overlap, most *C. digitalis* and *C. scabra* are above the majority of the predator population. Encounters of *Freemania* with these limpets are presumably far fewer than with *Notoacmea scutum* and there may be correspondingly less pressure for the development of defensive mechanisms against this predator. Similar arguments have been presented to explain differences in responsiveness of *Collisella digitalis, C. scabra*, and *Notoacmea scutum* to predatory seastars (Bullock, 1953; Feder, 1963; Margolin, 1964b).

Vertical position in the intertidal zone carries with it a constellation of other pertinent influences, many of which relate to the increased severity of the physical environment higher in the intertidal zone. Certainly both high and low intertidal animals must deal both with physical and predatory pressures, but the magnitudes of the pressures differ. The balance between physical and predatory stresses may be expected to shift with intertidal height. Animals living in the high intertidal often emphasize mechanisms that give them additional protection from the physical environment and desiccation. *Collisella digitalis* secretes a protective ring of mucus around its mantle edge when exposed, and *C. scabra* occupies a home scar (Wolcott, 1973). Both may remain inactive for days at a time. It would seem less advantageous for these limpets to abandon their protective sites in headlong flight than it would for *Notoacmea scutum*, which lives lower in the intertidal zone and does not face the same degree of potential physiological stress. It is also noteworthy that *Collisella scabra*, which seems fully committed to one relatively permanent home scar (Sutherland, 1970), responds even less to *Freemania* than does *Collisella digitalis*, which may move from site to site within a broad home range (Frank, 1964).

Occupation of a home scar by *C. scabra* also may affect its behavior in response to predators independent of the influences of physiological stresses. While homing undoubtedly provides protection from desiccation, it may also provide an effective defense against some predators. The tight fit of the shell of *C. scabra* against the

walls of its home scar may reduce the probability that a limpet would be detected by a foraging flatworm and, if detected, successfully engulfed. If the home site is indeed an effective deterrent to flatworm predation, the absence of overt defensive responses such as mushrooming and flight would benefit *C. scabra*. The "no response" behavior of *C. scabra* to contact with *Freemania* should not be viewed as some unfortunate deficiency of this particular limpet, but is more likely the most appropriate response in the context of the total lifestyle of the limpet.

Regardless of what specific forces combine to determine the relative responsiveness of these limpets to *Freemania*, our data suggest that the more responsive limpet may be escaping predatory encounters more frequently. Twice, *Freemania* was found feeding on medium-sized specimens of the high intertidal *Collisella digitalis* even though the vertical distributions of *Freemania* and this limpet overlap little. In contrast, *Freemania* was seen feeding on *Notoacmea scutum* only once (a small specimen) even though the distributions of predator and prey overlap broadly and many encounters were observed. Although these data are fragmentary, they suggest a parallel with Feder's (1959, 1963) work on limpet predation by the seastar *Pisaster ochraceus* (Brandt). *Collisella scabra*, which is unresponsive to *Pisaster ochraceus*, was the most abundant limpet in the diet of *P. ochraceus* even though the distributions of this seastar and *Collisella scabra* overlapped very little. In contrast, limpets such as *Notoacmea scutum*, which possessed vigorous defensive responses but also overlapped broadly with the predator, were found relatively infrequently in the predator's diet.

The scope and significance of marine flatworms as predators on gastropods are poorly known. Frank (1965) reports repeatedly seeing a polyclad feeding on *Collisella digitalis* in Oregon, and Hyman (1951; p. 200) lists snails as one of many food items of larger flatworms. Heath & McGregor (1912) report finding the large marine polyclad *Alloioplana californica* with gastropod radulae in the gut, but it is not known whether the gastropods were dead or alive when consumed. This small number of published reports is certainly due in part to difficulties of observing and identifying marine flatworms in the field. Flatworms tend to avoid light; and during daylight low tides, they are likely to be inactive and out of sight under rocks or in crevices. Once discovered, marine polyclads are difficult to identify. Even tentative species identification often has to be based on details of the reproductive apparatus and the arrangement of microscopic ocelli (Hyman, 1953).

Although reports of polyclad predation on gastropods are at present few, we suggest that such predation is not insignificant. *Freemania* is one of the more common flatworms from central California to British Columbia, and *Alloioplana californica* is common along the entire coast of California (Hyman, 1953). Within this broad geographical range, predation can be intense. Frank (1965) described a "trail of limpet shells" left by predatory polyclads, some of which had ingested as many as five snails. Furthermore, while predation by flatworms is undoubtedly

most intense on small limpets, we have seen flatworms eating limpets that were up to 1/3 the length of the predator. The maximum reported length for *Freemania* is 40 mm, as it is for *Alloioplana californica* (Hyman, 1953), and even the largest specimens of *Notoacmea scutum* tested (28 mm) responded to contact with *Freemania*. Finally, polyclads may have more time available for foraging than some other invertebrate predators. *Freemania* can forage during periods of exposure as well as during periods of submergence, whereas foraging by intertidal seastars, for instance, generally is limited to periods of submergence.

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## References

Ansell, A.D., 1969. Defensive adaptations to predation in the Mollusca. *Proc. mar. biol. Ass. India, Symp. Series*, Vol. 3, pp. 487–512.

Boone, E.S., 1929. Five new polyclads from the California coast. *Ann. Mag. Nat. Hist. (Ser. 10)*, Vol. 3, pp. 33 46.

Branch, G.M., 1979. Aggression by limpets against invertebrate predators. *Anim. Behav.*, Vol. 27, pp. 408–410.

Bullock, T.H., 1953. Predator recognition and escape responses of some intertidal gastropods in presence of starfish. *Behaviour*, Vol. 5, pp. 130–140.

Edwards, D.C., 1969. Predators on *Olivella biplicata*, including a species-specific predator avoidance response. *Veliger*, Vol. 11, pp. 326–333.

Ffder, H.M., 1959. The food of the starfish, *Pisaster ochraceus*, along the California coast. *Ecology*, Vol. 40, pp. 721–724.

Feder, H.M., 1963. Gastropod defensive responses and their effectiveness in reducing predation by starfishes. *Ecology*, Vol. 44, pp. 505–512.

Feder, H.M., 1972. Escape responses in marine invertebrates. *Sci. Am.*, Vol. 227, pp. 92–100.

Frank, P.W., 1964. On home range of limpets. *Am. Nat.*, Vol. 98, pp. 99–104.

Frank, P.W., 1965. The biodemography of an intertidal snail population. *Ecology*, Vol. 46, pp. 831–844.

Freeman, D., 1933. The polyclads of the San Juan region of Puget Sound. *Trans. Am. microsc. Soc.*, Vol. 52, pp. 107–146.

Froehlich, C.G., 1955. On the biology of land planarians. *Zool. Biol. Mar. (Sao Paulo)*, Vol. 20, pp. 263–271.

Heath, H. & E.A. McGregor, 1912. New polyclads from Monterey Bay, California. *Proc. Acad. nat. Sci. Philad.*, Vol. 64, pp. 455–488.

Hyman, L.H., 1951. *The invertebrates: Platyhelminthes and Rhynchocoela*. Vol. 2, McGraw-Hill, New York, 550 pp.

Hyman, L.H., 1953. The polyclad flatworms of the Pacific coast of North America. *Bull. Am. Mus. nat. Hist.*, Vol. 100, pp. 265–392.

Koopowitz, H., D. Silver & G. Rose, 1976. Primitive nervous systems. Control and recovery of feeding behavior in the polyclad flatworm, *Notoplana acticola*. *Biol. Bull. mar. biol. Lab., Woods Hole*, Vol. 150, pp. 411–425.

MacGinitie, G.E. & N. MacGinitie, 1968. *Natural history of marine animals*. McGraw-Hill, New York, 523 pp.

MACKIE, A.M. & P.T. GRANT, 1974. Interspecies and intraspecies chemoreception by marine invertebrates. In, *Chemoreception in marine organisms*, edited by P.T. Grant & A.M. Mackie, Academic Press, London, pp. 105–141.

MARGOLIN, A.S., 1964a. The mantle response of *Diodora aspera*. *Anim. Behav.*, Vol. 12, pp. 187–194.

MARGOLIN, A.S., 1964b. A running response of *Acmaea* to seastars. *Ecology*, Vol. 45, pp. 191 193.

PHILLIPS, D.W., 1975. Distance chemoreception-triggered avoidance behavior of the limpets *Acmaea (Collisella) limatula* and *Acmaea (Notoacmea) scutum* to the predatory starfish *Pisaster ochraceus*. *J. exp. Zool.*, Vol. 191, pp. 199–210.

PHILLIPS, D.W., 1976. The effect of a species-specific avoidance response to predatory starfish on the intertidal distribution of two gastropods. *Oecologia*, Vol. 23, pp. 83 94.

PHILLIPS, D.W., 1977. Avoidance and escape responses of the gastropod mollusc *Olivella biplicata* (Sowerby) to predatory asteroids. *J. exp. mar. Biol. Ecol.*, Vol. 28, pp. 77–86.

RICKETTS, E.F., J. CALVIN & J.W. HEDGPETH, 1968. *Between Pacific tides*. Stanford University Press, Stanford, 614 pp.

SUTHERLAND, J.P., 1970. Dynamics of high and low populations of the limpet, *Acmaea scabra* (Gould). *Ecol. Monogr.*, Vol. 40, pp. 169–188.

THUM, A.B., 1974. Reproductive ecology of the polyclad turbellarian *Notoplana acticola* (Boone, 1929) on the central California coast. In, *Biology of the Turbellaria*, edited by N.W. Riser & M.P. Morse, McGraw-Hill, New York, pp. 431–445.

WOLCOTT, T.G., 1973. Physiological ecology and intertidal zonation in limpets (*Acmaea*): a critical look at "limiting factors." *Biol. Bull. mar. biol. Lab., Woods Hole*, Vol. 145, pp. 389–422.

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# NITRATE AND NITRITE IN INTERSTITIAL WATERS OF EELGRASS BEDS IN RELATION TO THE RHIZOSPHERE

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**Abstract:** The distributions of nitrate and nitrite in the interstitial water of the sediment of eelgrass (*Zostera marina*) bed of Izembek Lagoon, Alaska, were investigated. Their concentrations were relatively high (0 to 9.8 $\mu$g-at.N $\cdot$ l$^{-1}$, average 4.8 for nitrate; 0 to 4.0 $\mu$g-at.N $\cdot$ l$^{-1}$, average 1.9 for nitrite) although the sediments were anoxic and contained hydrogen sulphide. The rates of bacterial denitrification measured by $^{15}$N tracer technique ranged from $0.49 \times 10^{-10}$ to $1.2 \times 10^{-9}$ g-at.N $\cdot$ g$^{-1}$ $\cdot$ h$^{-1}$. When a steady state is maintained, the loss of nitrate and nitrite must be balanced by their production by bacterial nitrification. Experimentally determined rate of nitrification in the sediment was of the same order. A model experiment demonstrated that oxygen is transported from leaves to rhizomes and roots of eelgrass and released into the sediment. The oxygen is used for nitrification in the rhizosphere in anoxic sediments.

## INTRODUCTION

The distribution of nitrate and nitrite and bacterial denitrification in sediments have been studied by many workers (Goering & Pamatmat, 1971; Vanderborght & Billen, 1975; Bender *et al.*, 1977; Grundmanis & Murray, 1977; Sørensen, 1978; Oren & Blackburn, 1979). Nitrate and nitrite, in general, are restricted in an oxidized zone, and denitrification is most intensive at the boundary between oxidized and reduced zones. High concentrations of nitrate and nitrite were, however, detected in a reduced sediment of an eelgrass (*Zostera marina*) bed of Mangoku-Ura, north-eastern Japan (Iizumi, 1975). Occurrence of denitrification at this site was demonstrated by Koike & Hattori (1978).

The present paper reports the distribution of nitrate and nitrite and the occurrence of denitrification in deep (20 cm) hydrogen sulphide-containing sediments of an eelgrass bed of Izembek Lagoon, Alaska. Experimental evidence is presented which shows an oxygen transport system in eelgrass. Oxygen evolved within its foliar tissues in association with photosynthesis is transported to rhizomes and roots through the lacunal system of the plants and released into sediment. This system would provide oxygen necessary for nitrification which probably occurs in eelgrass rhizosphere in anoxic sediments.

## MATERIALS AND METHODS

### SAMPLING SITE

Sampling and experiments were done from July to August 1977, at Izembek Lagoon, which is located at the western end (55°15′N:163°50′W) of Alaska Peninsula. The lagoon is 41 km long and 3 to 12 km wide, more than half of which is covered by eelgrass populations (McRoy, 1970).

Fig. 1 shows a transect of the site studied. Stations 1 to 4 were in an intertidal zone, where the eelgrass was exposed to the air at low tide. At Station 1, eelgrass populations formed colonizing patches, and had short ( < 30 cm) and narrow ( < 2 mm) leaves; the standing stock was the lowest among the seven stations examined. Eelgrass populations at Station 4 had the same type of leaves as at Station 1, but they uniformly covered the bottom, so had the highest shoot density. Station 10 was in a subtidal zone where eelgrass populations had long ( > 1 m) and wide ( > 2 mm)

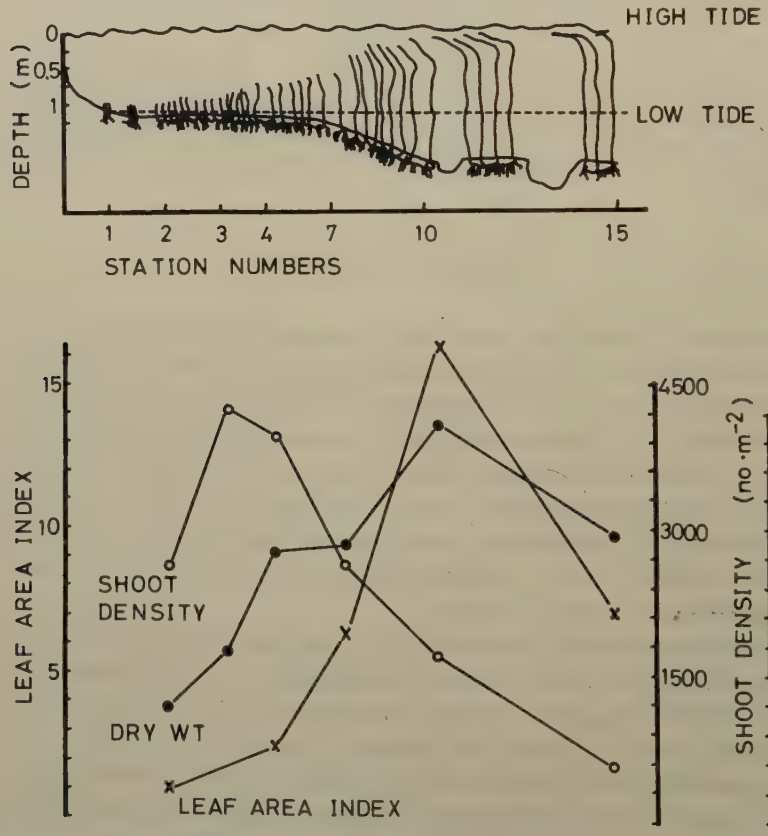


Fig. 1. Leaf area index, shoot density, and dry weight of eelgrass along a transect of Izembek Lagoon, Alaska, July 1977.

leaves. The standing stock and leaf area index (LAI) were highest at Station 10. Sediments consisted of sandy mud at Stations 1 and 4, and of soft mud rich in organic detritus at Station 10.

### INTERSTITIAL WATER ANALYSIS

Sediment samples were taken with Plexiglas tubes (diameter, 5 to 7 cm). Interstitial water was extracted with a gas-operated squeezer as described by Reeburgh (1967) and stored frozen for later determination of nitrate and nitrite.

Nitrate and nitrite were determined by a Technicon AutoAnalyzer, using the method of Morris & Riley (1963) and the method of Bendschneider & Robinson (1952) modified for an autoanalyzer, respectively. Before analysis, hydrogen sulphide was removed by the following procedure of I. Koike (unpubl.): an equal volume of 0.1 M $HgCl_2$ was added to the sample; after standing for 4 days the precipitate of HgS was removed by centrifugation.

Ammonium was determined by the method of Koroleff (1976) after dilution with de-ionized water.

### MEASUREMENT OF DENITRIFICATION ACTIVITY

Denitrification was measured by a $^{15}N$ tracer technique (Goering & Dugdale, 1966). The rate was calculated from the increase of $^{15}N$ labelled nitrogen with time in a series of five duplicate incubation tubes whose biological reactions were terminated after different times (0 to 40 h maximum).

After sediment samples, obtained from the same depth of three cores, were combined, 1 g (wet wt) portions of subsamples were placed in each tube (vol. 20 ml). The tubes were filled with sea water which had been filtered through a Millipore HA filter and bubbled with nitrogen for at least 30 min to eliminate dissolved oxygen. The tubes were capped with rubber stoppers. $^{15}N$ labelled $KNO_3$ (50 atom % $^{15}N$) was added with a hypodermic syringe so as to give final concentration of nitrate approximately equal to the in situ concentration (1.1 to 9.1 $\mu$g-at.N $\cdot$ $l^{-1}$). Care was taken not to leave air bubbles in the tubes. The tubes were incubated horizontally, so that diffusion of nitrate into sediment did not limit the activity, and kept at about in situ temperature (11 to 15 °C). Biological reactions were terminated by injecting $HgCl_2$ (2.5 ml of 0.1 M $HgCl_2$ to each tube) with a syringe. The tubes were stored at room temperature until isotope ratio determination.

Dissolved nitrogen was quantitatively extracted by the aid of sonication and introduced into an inlet system of a Hitachi RMU-6E mass spectrometer fitted with a dual inlet-double collector system and a digital integrator. Denitrification rates were calculated on the assumption that the excess of $^{15}N$ in nitrogen was derived from $^{15}N$ labelled $KNO_3$ by bacterial denitrification.

MEASUREMENT OF NITRIFICATION ACTIVITY

Five g (wet wt) of sediment sample were suspended in 600 ml of sea water which had been filtered through a Gelman type AE glass fiber filter. $NH_4Cl$ was added so as to give a final concentration of ammonium of 400 $\mu$g-at.N $\cdot$ $l^{-1}$. Incubation was conducted at 15 °C under aerobic conditions. At intervals, 50 ml of aliquots were removed and nitrate and nitrite concentrations were determined.

MEASUREMENT OF OXYGEN RELEASE FROM THE ROOT–RHIZOME SYSTEM

Eelgrass samples collected at Nabeta Bay (34°40′N:138°55′E) in January 1979 and maintained in an aquarium for 3 wk were used for the oxygen release experiment. The experimental apparatus consisted of three chambers made of Plexiglas: a root chamber, a plant chamber, and a thermostat (Fig. 2). A rhizome with roots was enclosed in the root chamber (vol. 36.4 ml), fitted with a Clark type oxygen electrode (Yellow Spring, YSI 4004). The root chamber was filled with a Millipore HA filtered sea water, which had been bubbled with nitrogen to reduce oxygen content to

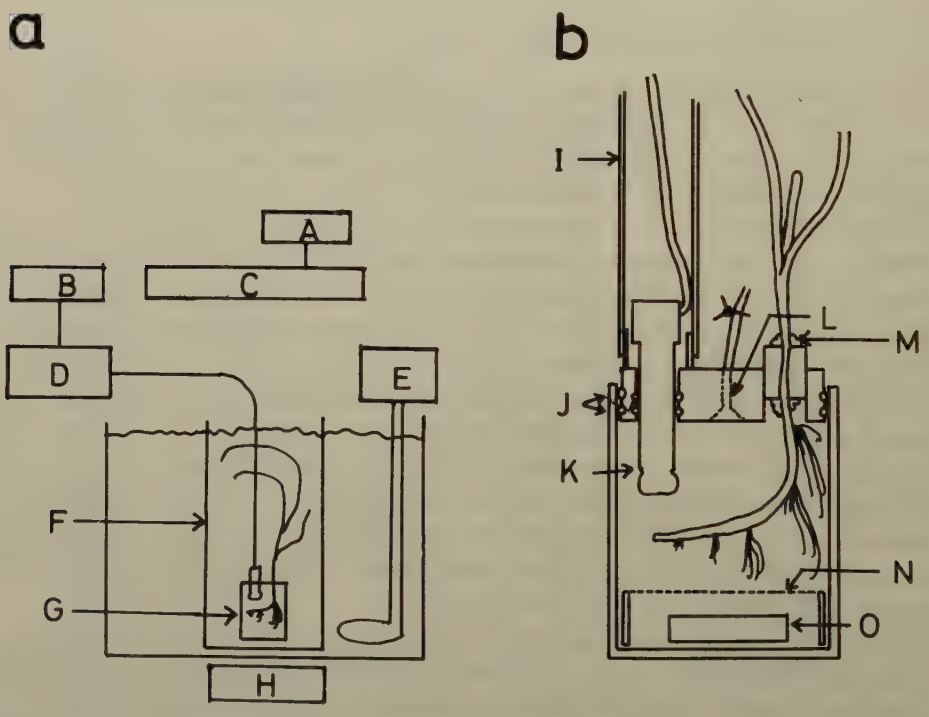


Fig. 2. Apparatus for measurement of oxygen release from the root–rhizome system of eelgrass: a, the whole system; b, details of the root chamber; A, timer; B, recorder; C, fluorescent lamps; D, power supply for the electrode; E, temperature controller; F, plant chamber; G, root chamber; H, stirrer; I, tube to protect the cord from water; J, "o" rings; K, oxygen electrode; L, hole for de-bubbling; M, silicone grease; N, net to protect roots from the stirring bar; O, stirring bar.

≈ 10% of air saturation, and covered with aluminium foil to reduce light. The whole plant was placed in the plant chamber filled with filtered sea water. Oxygen content was continuously monitored at 12 °C. The plant was illuminated by fluorescent lamps intermittently at 3-h intervals. The average intensity of light in the plant chamber was ≈ 6000 lux.

## Results

### Distribution of nitrate and nitrite in interstitial waters

Fig. 3 shows vertical profiles of nitrate and nitrite in interstitial waters of the eelgrass bed of Izembek Lagoon. Concentrations of nitrate and nitrite were invariably higher than those in overlying water. The highest concentration of nitrate (9.8 $\mu$g-at.N $\cdot l^{-1}$) was observed in the 7- to 10-cm layer of Station 10. Even below 30 cm,

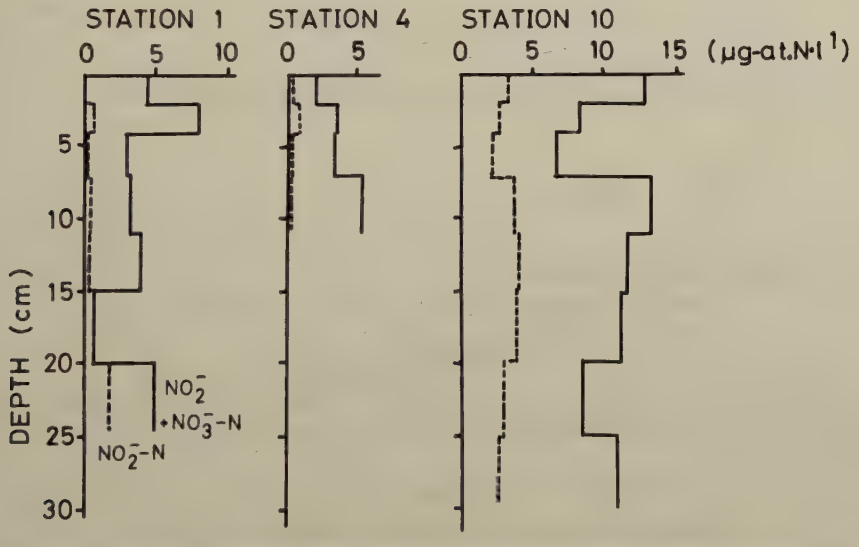


Fig. 3. Vertical profiles of nitrate and nitrite in interstitial waters of eelgrass bed sediment of Izembek Lagoon, Alaska, July 1977.

≈ 8 $\mu$g-at.N $\cdot l^{-1}$ of nitrate was detected. Nitrite was also highest at this station. The average concentrations of nitrate plus nitrite over 0 to 30 cm were 10 $\mu$g-at. N $\cdot l^{-1}$ at Station 10, 3.8 at Station 4, and 3.7 at Station 1.

### Denitrification

Fig. 4 shows time courses of $^{15}N$ labelled nitrogen production from $^{15}N$ labelled $KNO_3$ when sediments from Station 10 were incubated under anaerobic conditions. Nitrogen production proceeded linearly with time for at least 22 h irrespective of

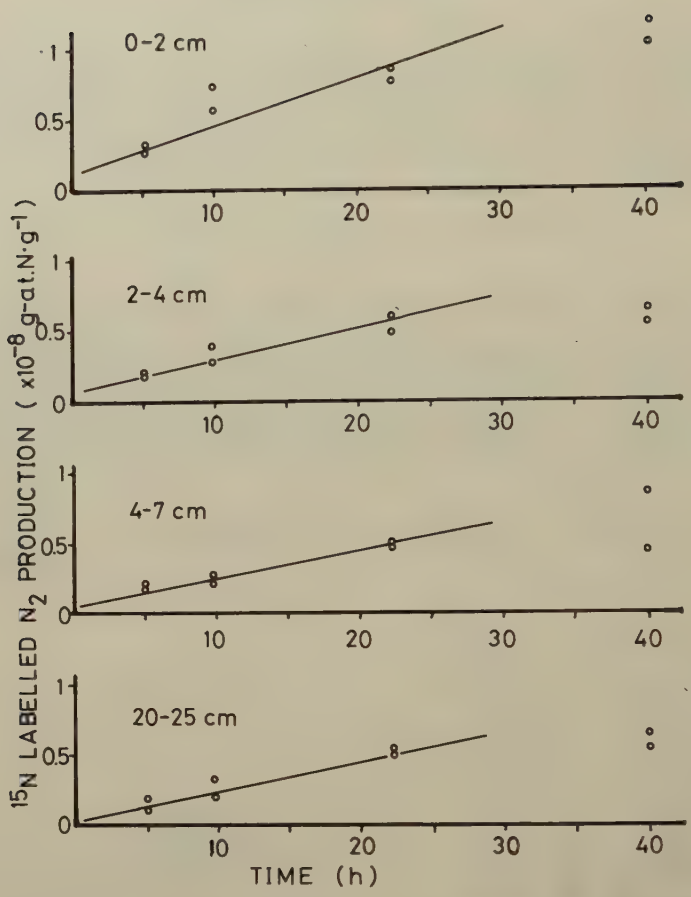


Fig. 4. Time courses of $^{15}N$ labelled $N_2$ production from $^{15}N$ labelled $KNO_3$ by sediments of various layers of Station 10; regression lines fitted for the initial 22-h incubation period are also shown.

TABLE I

Denitrification in eelgrass bed sediments of Izembek Lagoon, Alaska.

| Station | Depth (cm) | Denitrification activity* (g-at.N · g$^{-1}$ · h$^{-1}$) |
|---|---|---|
| 1 | 0–2 | $1.3 \times 10^{-10}$ |
| | 2–4 | $0.71 \times 10^{-10}$ |
| | 4–7 | $1.5 \times 10^{-10}$ |
| | 20–24 | $0.79 \times 10^{-10}$ |
| 4 | 0–2 | $0.49 \times 10^{-10}$ |
| | 2–4 | $0.80 \times 10^{-10}$ |
| | 4–7 | $1.0 \times 10^{-10}$ |
| 10 | 0–2 | $1.2 \times 10^{-9}$ |
| | 2–4 | $5.2 \times 10^{-10}$ |
| | 4–7 | $3.6 \times 10^{-10}$ |
| | 20–25 | $5.3 \times 10^{-10}$ |

* See text for calculation of denitrification activity at in situ nitrate concentrations.

sediment depth. A Lineweaver-Burk plot of results yielded a value of 53 $\mu$g-at.N $\cdot$ l$^{-1}$ of nitrate for $K_m$ with the surface (0–3 cm) sediment of Station 4. In situ concentrations of nitrate were much lower than this. According to Koike & Hattori (1978) the rate of $N_2$ production from nitrite is almost identical with that from nitrate. In situ denitrification rates can, therefore, be calculated simply by applying a proportional relationship between the sum of nitrate and nitrite concentrations and a denitrification activity (Table I).

The rates obtained from Izembek Lagoon are in the same range as those reported by Koike & Hattori (1978) and by Oren & Blackburn (1979). Average in situ denitrification over 0 to 7 cm at Station 10 ($6.9 \times 10^{-10}$ g-at.N $\cdot$ g$^{-1}$ $\cdot$ h$^{-1}$) was higher than at other stations ($1.2 \times 10^{-10}$ g-at.N $\cdot$ g$^{-1}$ $\cdot$ h$^{-1}$ at Station 1, and $0.69 \times 10^{-10}$ g-at.N $\cdot$ g$^{-1}$ $\cdot$ h$^{-1}$ at Station 4).

## NITRIFICATION

Nitrate and nitrite increased with time linearly when sediment samples were incubated in the presence of 400 $\mu$g-at.N $\cdot$ l$^{-1}$ of ammonium under aerobic conditions (Fig. 5). After 27.5 h, 0.57 and 0.56 $\mu$g-at.N $\cdot$ l$^{-1}$ of nitrate plus nitrite were produced

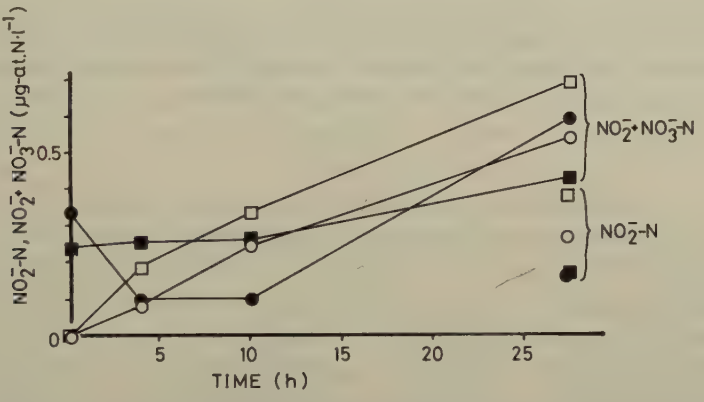


Fig. 5. Changes with time in concentrations of nitrate plus nitrite in media when sediments from two layers (○, ● for 0–3 cm; and □, ■ for 3–6 cm; duplicates) of Station 4 were incubated with 400 $\mu$g-at.N $\cdot$ l$^{-1}$ of ammonium: concentrations of nitrite after 27.5 h are also shown.

for 0–3 and 3–6 cm sediments, respectively. The average of four determinations gave a value of $2.7 \times 10^{-9}$ g-at.N $\cdot$ g$^{-1}$ $\cdot$ h$^{-1}$ for nitrification. This rate is three to five times higher than denitrification rates of the same layer. Taking into account in situ ammonium concentrations (10 to 130 $\mu$g-at.N $\cdot$ l$^{-1}$, C. P. McRoy & M. J. Klug, unpubl.), the rate of in situ nitrification would be lower and of the same order as that of denitrification.

## OXYGEN RELEASE FROM THE ROOT-RHIZOME SYSTEM

When an eelgrass shoot was kept in the dark, the oxygen content in the root chamber decreased with time (Fig. 6). This can be attributed to respiration of the plant. The oxygen content started to increase within 30 min after the light was switched on. The rate of transport from leaves to roots and rhizomes can be calculated as the difference between the rates of oxygen content variation in the light and in the dark (Table II). About 50 to 60% of oxygen transported to the roots and rhizomes was released from the plant system.

Fig. 6. Change with time in oxygen concentration in the root chamber (Fig. 2) as measured by an oxygen electrode: 1 mV of electrode output was equivalent to 9.9 $\mu$mol $O_2 \cdot l^{-1}$; light was switched on or off at times indicated by arrows; initial rates in the light and in the dark were used for calculation of oxygen release rate presented in Table II.

TABLE II

Oxygen release from the root-rhizome system of eelgrass.

| | | Unit | Expt. 1 | Expt. 2 |
|---|---|---|---|---|
| Dry wt of roots and rhizome | (A) | mg | 36 | 54 |
| Dry wt of leaf | (B) | mg | 78 | 133 |
| Respiration rate | (C) | $\mu$mol $O_2 \cdot h^{-1}$ | −0.65 | −1.30 |
| Respiration rate per unit wt | (C/A) | $\mu$mol $O_2 \cdot mg^{-1} \cdot h^{-1}$ | −0.018 | −0.024 |
| Oxygen release rate | (D) | $\mu$mol $O_2 \cdot h^{-1}$ | +0.97 | +1.44 |
| Oxygen transport rate* | (D − C = E) | $\mu$mol $O_2 \cdot h^{-1}$ | +1.62 | +2.74 |
| Oxygen transport rate per unit wt | (E/B) | $\mu$mol $O_2 \cdot mg^{-1} \cdot h^{-1}$ | +0.021 | +0.021 |

* See text.

When a rhizome of eelgrass was squeezed underwater small bubbles were produced; microscopic observation showed gas lacunae in rhizomes connected to those of leaves. It is likely that oxygen is transported from leaves to rhizomes and roots through this lacunal system. The exact site of release is unknown.

## Discussion

In sediment systems bacteria oxidize organic matter using, at first, oxygen dissolved in interstitial water. When the oxygen is used, nitrate and nitrite are used as electron acceptors; after their exhaustion sulphate reduction is initiated. The occurrence of sulphide, the product of sulphate reduction, therefore indicates that no oxygen, nitrate or nitrite is left for bacterial respiration.

Two types of distribution have been reported with respect to nitrate and nitrite: (1) sea water above sediment contains nitrate (and nitrite), which diffuses into sediment and is denitrified (Goering & Pamatmat, 1971), and (2) nitrification occurs in the surface sediment, and nitrate (and nitrite) produced there diffuses into a reduced layer below (Billen, 1976). In the former case, nitrate simply decreases with depth in sediment, and in the latter case a maximum of nitrate (and nitrite) appears below the surface of the sediment.

In the seagrass beds, sediments are rich in organic detritus, which mainly consist of dead leaves, rhizomes, and roots of seagrass. Therefore, except for the top few millimeters of the sediment a reduced zone is well developed as indicated by a black colour together with the odour of hydrogen sulphide. Nitrate and nitrite, however, were detected in interstitial waters of the eelgrass bed sediments of Mangoku-Ura (Iizumi, 1975). Their concentrations were nearly 10 times higher in the top 25 cm of sediment than those in overlying water. A similar situation was found in the eelgrass beds of Izembek Lagoon. Hydrogen sulphide (50 to 800 $\mu$g $H_2S$-S $\cdot l^{-1}$, C. P. McRoy & M. J. Klug, unpubl.) was found in the sediments where nitrate and nitrite were detected.

When a steady state is maintained in the sediment with respect to nitrate and nitrite, their loss must be balanced by their production (nitrification). Molecular oxygen is required for nitrification. Grundmanis & Murray (1977) suggested that the irrigation by benthic organisms supplies the oxygen necessary for nitrification in deep (20–30 cm) sediment. Oremland & Taylor (1977) reported a diurnal fluctuation of oxygen content in bubbles from the sediment of a *Thalassia testudinum* bed and discussed the possibility that oxygen was supplied to the sediment through the root–rhizome system of the seagrass. Our data provide direct evidence of the oxygen transport system of eelgrass. The oxygen which is produced by photosynthesis in leaves is transported to the rhizomes and roots through gas lacunae. A part of the oxygen is consumed by respiration within root and rhizome tissues but the rest is released through cell membranes. Whether eelgrass actively diffuses oxygen around its roots and rhizomes to protect them from hostile environments or extra oxygen diffuses out through cell membranes is unknown. The latter case seems more likely. In any event, nitrifying bacteria around roots and rhizomes can oxidize ammonium to nitrite and nitrate using the released oxygen. A large part of the oxygen is probably used by other bacteria for oxygen respiration. The supply of oxygen would be limited within the rhizosphere of the eelgrass, so the majority of sediment remains anoxic,

where bacterial denitrification proceeds using nitrate and nitrate provided from the rhizosphere.

Both the standing stock of eelgrass and organic matter content in the sediment were higher at Station 10 than at Station 4. On the contrary, unpublished data of C.P. McRoy & M.J. Klug showed that concentrations of hydrogen sulphide in sediment were two to eight times higher at Station 4 than at Station 10. This can be explained by the difference in oxygen supply to sediments through eelgrass between the two stations. The amount of oxygen supplied into sediments by the oxygen transport system of eelgrass is greater at Station 10 because of higher LAI. The oxygen is preferably utilized over sulphate for decomposition of organic matter, so less sulphide is produced.

The eelgrass bed of Shimoda Bay, next to Nabeta Bay where the samples for oxygen release experiment were collected, had 220 $g \cdot m^{-2}$ of leaf biomass in May 1974 (Iizumi, 1975). Biomass of the underground portion was 37% of leaf biomass. Using the value given in Table II, and assuming a day length of 12 h, the oxygen released by eelgrass into sediment is calculated to be 35 mmol $O_2 \cdot m^{-2} \cdot day^{-1}$. The in situ denitrification rate is $\approx 1$ ng-at.$N \cdot g^{-1} \cdot h^{-1}$ (I. Koike, unpubl.). Assuming that nitrification occurs in top 15 cm layer of sediment where eelgrass roots extend, at the same rate as denitrification, we obtain a value of 2.2 mmol $O_2 \cdot m^{-2} \cdot day^{-1}$ for the oxygen requirement. This value is $\approx 6\%$ of the amount transported by eelgrass. Although many assumptions are included in the calculation, it illustrates the importance of the root–rhizome system of eelgrass in nitrogen cycling, as well as in other bacterial and chemical reactions in coastal sediments.

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## References

Bender, M.L., K.F. Fanning, P.N. Froelich & V. Maynard, 1977. Interstitial nitrate profiles and oxidation of sedimentary organic matter in the eastern equatorial Atlantic. *Science*, Vol. 198, pp. 605–609.

Bendschneider, K. & R.J. Robinson, 1952. A new spectrophotometric method for the determination of nitrite in sea water. *J. mar. Res.*, Vol. 11, pp. 87–96.

Billen, G., 1976. Evaluation of nitrifying activity in sediments by dark $^{14}C$-carbonate incorporation. *Water Res.*, Vol. 10, pp. 51–57.

GOERING, J. J. & V. A. DUGDALE, 1966. Estimates of the rates of denitrification in a subarctic lake. *Limnol. Oceanogr.*, Vol. 11, pp. 113–117.

GOERING, J. J. & M. M. PAMATMAT, 1971. Denitrification in sediments of the sea off Peru. *Investigacion pesq.*, Vol. 35, pp. 233–242.

GRUNDMANIS, V. & J. W. MURRAY, 1977. Nitrification and denitrification in marine sediments from Puget Sound. *Limnol. Oceanogr.*, Vol. 22, pp. 804–813.

IIZUMI, H., 1975. Dynamics of inorganic nitrogen in eelgrass beds. M.S. thesis, University of Tokyo, Tokyo, Japan, 73 pp.

KOIKE, I. & A. HATTORI, 1978. Denitrification and ammonium formation in anaerobic coastal sediments. *Appl. Environ. Microbiol.*, Vol. 35, pp. 278–282.

KOROLEFF, F., 1976. Determination of $NH_4$ -N. In, *Methods of seawater analysis*, edited by K. Grasshoff, Verlag Chemie, Weinheim, pp. 127–133.

MCROY, C. P., 1970. On the biology of eelgrass in Alaska. Ph.D. dissertation, University of Alaska, Fairbanks, U.S.A., 156 pp.

MORRIS, A. W. & J. P. RILEY, 1963. The determination of nitrate in seawater. *Analytica chim. Acta*, Vol. 29, pp. 272–279.

OREMLAND, R. S. & B. F. TAYLOR, 1977. Diurnal fluctuations of $O_2$, $N_2$ and $CH_4$ in the rhizosphere of *Thalassia testudinum*. *Limnol. Oceanogr.*, Vol. 22, pp. 566–570.

OREN, A. & T. H. BLACKBURN, 1979. Estimation of sediment denitrification rates at *in situ* nitrate concentrations. *Appl. Environ. Microbiol.*, Vol. 37, pp. 174–176.

REEBURGH, W. S., 1967. An improved interstitial water sampler. *Limnol. Oceanogr.*, Vol. 12, pp. 163–165.

SØRENSEN, J., 1978. Denitrification rates in marine sediment as measured by the acethylene inhibition technique. *Appl. Environ. Microbiol.*, Vol. 36, pp. 139–143.

VANDERBORGHT, J.-P. & G. BILLEN, 1975. Vertical distribution of nitrate concentration in interstitial water of marine sediments with nitrification and denitrification. *Limnol. Oceanogr.*, Vol. 20, pp. 953–961.

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